EXPERIMENTAL RESEARCHES WITH THE MICROBES OF CHICKEN-CHOLERA.

By Dr. Oscar Katz.

Introduction.

It will be remembered that Pasteur recommended, as a means for rabbit-extermination on a large scale, the disease commonly known under the name of *choléra des poules*, chicken- or fowl-cholera. The Royal Intercolonial Commission, appointed in April last year by the Australasian Governments to inquire into, and report upon, the schemes submitted for the extermination of rabbits in Australasia—a prize of £25,000 being offered for a successful remedy by the New South Wales Government—at once took the necessary steps to make itself acquainted with Pasteur's proposal. Being, however, dissatisfied with the information already to hand about the merits of this particular disease, or rather the microbes of this disease, as rabbit-exterminators, and considering the results of the experiments performed in France by Pasteur or under his direction, and of those by his delegates in Sydney, as unsatisfactory, it decided to have experiments of its own carried out.

As chief expert officer to the Commission, I was entrusted with this work. A laboratory—intended also for the investigation of any other scheme that might be worthy of consideration—was built on an hitherto unoccupied islet, called Rodd Island, in Iron Cove (Leichhardt Bay), a western portion of Port Jackson. The little island, of solid sandstone, and covered here and there with scrub, was well adapted for the object in view. Its plateau was mostly formed of loose sandy soil. The laboratory, a substantial building

of corrugated galvanised iron, the space between the sheets of the walls being filled up with sawdust in addition to a lining of felt, contained four rooms, and was fitted out with what appeared necessary. Gas was produced on the Island itself, out of gasolene, through a Müller's "Alpha Gas Making Machine," ordinary coal-gas across the water not being obtainable.* Water-pipes were laid on, the water supplied being rain-water collected in iron tanks. On one free side of the laboratory, under the verandah, arrangements were made for accommodating a large number of rabbits.

In the centre was erected a large enclosure, covered in all over with fly-proof wire-gauze in connection with wide-meshed wire-netting. This enclosure measured 100 feet (about $30\frac{1}{2}$ mètres) in length, and 80 feet ($24\frac{3}{8}$ mètres) in width; it was slightly cut off at the corners. Most of the surface-area consisted of loose soil, in which artificial burrows could easily be dug (as will be seen later on); a small portion only being taken up by rocks (sandstone), which were partly on a level with the soil-surface, partly more or less projecting. There were a few small trees (gums, geebung) preserved in this enclosed place.

Adjoining one of the shorter sides of this main enclosure, was a large shed covered all round with corrugated iron, and having a brick-basement; at the rear of this shed was a number of pens and stalls.

In one corner of the Island, towards the water-edge, was an aviary, 15 feet (about $4\frac{1}{2}$ mètres) square. One, the southern, half of it was covered at top and sides with sheets of galvanised iron; the other, northern half, only with wide-meshed netting and fly-proof wire-gauze. The greater portion of the aviary was accessible to the sun for nearly all day.

A dwelling-house, with belongings, completed the collection of buildings on Rodd Island.

---

* See my communication "On 'Air-gas' for Bacteriological Work;" these Proceedings, Vol. IV. (2nd Ser.), p. 328.

The following pages contain an account of my researches with regard to the microbes of chicken-cholera. These researches, as far as they were carried out before April last, were made the subject of five Progress Reports laid before the Commission from time to time, and printed in the Volume of Proceedings of that Commission. I think that sufficient interest attaches to the subject to be dealt with in a scientific journal. For this purpose the whole available material, including that which was obtained since April last, has been worked up and grouped in an appropriate manner.

To Messrs. F. Dillon Bell and J. P. Meagher, who in succession were Assistants on Rodd Island, I am indebted for the services rendered by them in regard to the various experiments.

General Remarks.

The microbes with which all the experiments recorded in the following pages were carried out, were descended from those which were brought to Sydney from Paris by Pasteur's representatives. When, August 4th, 1888, the latter concluded their experiments of demonstration, which were begun about a month previously (July 7th), and to which attention has already been directed in the introduction (a special report on that demonstration may be found in the Volume of Proceedings of the Royal Commission), I took, with M. Loir's permission, some blood from the heart of a rabbit which had died after feeding on virulent broth-culture of the chicken-cholera microbes. Pure cultures were obtained from a "colony" on nutrient gelatine (after Esmarch's roll-method) from the blood of a rabbit, which had been inoculated with broth-culture in second generation, derived originally from the above-mentioned sample of blood.

In my experiments, partly such material was used as originated from that "colony," and was cultivated from tube to tube; partly cultures prepared directly from the heart-blood of rabbits newly dead from virulent "chicken-cholera," and not otherwise diseased. Such blood, as a rule, only contains the microbes under consideration.

As liquid medium for the cultivation of these microbes I employed rabbit-flesh infusion, in the following briefly termed rabbit-broth, or simply broth. Stated in a few words, this liquid was prepared by allowing finely minced flesh of well-nourished, thoroughly healthy, wild rabbits to stand with double the quantity (in weight) of distilled water, in a cool place, for twenty-four hours, stirring up from time to time, filtering and pressing through cheese-cloth, steaming, filtering again, neutralising with 20 p.c. watery solution of anhydrous carbonate of soda, or rather producing a slightly alkaline reaction, steaming and filtering again, and ultimately filling into different-sized, cotton-wool-plugged, sterilised test-tubes, which with their contents were thereupon discontinuously sterilised.

In such plain rabbit-broth, without any additional ingredients, the chicken-cholera bacteria grow very luxuriantly at a suitable temperature; they grow in that medium with pretty much the same vigour as in rabbit-broth to which 1 p.c. dry peptone and 0·5 p.c. sodium chloride are added. Broth of the latter description I employed, besides the former, in connection with certain experiments (*re* Immunisation, p. 526).

Of nutrient solid soils I mostly used a 6 p.c. rabbit-broth-peptone-gelatine, which was prepared in the usual way, with the difference that infusion of rabbit-flesh instead of beef-infusion was taken. On such a rabbit-broth-gelatine, the chicken-cholera microbes flourish excellently; fully developed stick-cultures always showed a substantial, expanded, superficial layer, of a whitish colour and sticky structure. The colour of the growth along the stick-canal, at first also whitish, changed into yellowish or yellowish-brown in old cultures; the same applied to isolated colonies in the gelatine.

In nutrient agar-agar—in the preparation of which beef-infusion was used—I saw the superficial growth (in stick-cultures) assume the shape of a thin film extending nearly over the whole surface, while the stick began to show by and by a darker coloration than the slightly yellow agar.

The usual nutrient gelatine (containing beef-infusion), as well as such gelatine with 2 p.c. grape sugar, or nutrient gelatine containing 2·7 p.c. sodium chloride, were occasionally taken into use.

In order to avoid repetitions, I will mention here that all the rabbits, upon which the microbes were tried from different points of view, were wild rabbits, if not specially noted to the contrary. These wild rabbits were ordered by the Rabbit Branch, Lands Department, Sydney, from near Hay, in New South Wales, about 420 miles from Sydney; they were mostly caught and sent to Rodd Island in a large number of consignments from Carrathool, near Hay. A few of the wild rabbits used came from Tasmania.

I ascertained the weight of six full-grown, perfectly healthy wild rabbits from Carrathool; the average weight was 1522 grammes (3 lbs. $5\frac{2}{3}$ oz.).*

Effect of Chicken-cholera Microbes on Rabbits.

It has been made known by Pasteur and others that rabbits manifest a great susceptibility towards the microbes of chicken-cholera, let the latter be applied as subcutaneous or cutaneous inoculation, through the alimentary canal, by way of injection into the peritoneal cavity, or of inhalation into the lungs. It has also been shown that the mucous surface of the uterus, after parturition, can form a means of entrance for the microbes, when

---

* In the Paper the terms cubic centimètre, gramme, centimètre, millimètre, centigrade (°Cels.), are often used. I give their English equivalents as follows:—

One (1) cubic centimètre (ccm.) = sixteen (16) minims (drops in general).

28·3495 grammes (g.) = 1 ounce.

1·7718 ,, = 1 dram.

2·539977 centimètres (cm.) / 25·39977 millimètres (mm.) = 1 inch.

n° Cels. (Centigr.) = 9/5 n + 32° Fahr.; [+20° C. = (9/5 × 20) + 32° F. = 68° F.]

other micro-organisms, highly pathogenic for rabbits, *e.g.*, anthrax bacilli, are powerless.*

Pasteur states that the action of chicken-cholera microbes is much more pronounced in the case of rabbits, than in that of fowls.† My observations in this direction—my material for experiments was derived from rabbits dead from "chicken-cholera"—are thus far in accordance with Pasteur's statement.

With regard to the effect of subcutaneous application of the microbes on rabbits, I can assert that the result which I obtained with *material of undoubted full virulence* (blood; artificial culture) on wild rabbits (also one tame one), *which had not been previously treated in any way*—I estimate the number of wild rabbits used in that way at about one hundred and fifty—was always a positive one. All of them succumbed to a disease which owed its origin to the chicken-cholera microbes. The time which it took from inoculation to death differed according to the degree of concentration of the virulent object introduced, and according to the individuality of the rabbits. Generally speaking, the microbes thus administered kill speedily. Instances hereof may be found in sufficient numbers later. The shortest time actually observed in a full-grown healthy specimen (♂), inoculated between the shoulder-blades with $\frac{1}{24}$ ccm. ($\frac{2}{3}$ minim) of heart-blood from a rabbit newly dead from "chicken-cholera," was about $8\frac{1}{2}$ hours; at another time, in the case of a half-grown rabbit inoculated at the belly with a small quantity of fresh broth-culture of the fourth generation, it was less than $7\frac{3}{4}$ hours. The longest space of time was observed in a full-grown, but apparently young doe, namely about forty-eight hours. This rabbit had been inoculated (at the belly) with $\frac{1}{40}$ ccm. of virulent rabbit-blood (see Table III., Rabbit No. 34; also p. 552).

---

* J. Straus et D. Sanchez-Toledo, "Recherches microbiologiques sur l'utérus après la parturition physiologique." *Annales de l'Institut Pasteur*, Tome II., No. 8, 1888, p 433.

† Sur la destruction des lapins en Australie et dans la Nouvelle-Zélande. *Annales de l'Institut Pasteur*, Tome II., No. 1, 1888, pp. 5-6.

My experience as to the effect which it has on fresh wild rabbits, when they are given to eat food contaminated with virulent chicken-cholera bacteria—this kind of treatment having naturally come largely and repeatedly into operation, as may be seen from the various experiments described in the following—may thus be summarised.

Small quantities of freshly prepared broth-cultures (1 ccm.-3 ccm.) of the microbes of chicken-cholera, or of blood derived from animals dead from the disease, added to food (green stuff, as cabbage- or barley-leaves; dry food, as bran) and consumed by fresh wild rabbits, caused the death of the animals with few exceptions. The time which, in this mode of infection, lay between feeding and death, fluctuated in the majority of instances between 18 and 25 hours; in others, more time elapsed until death followed; one full-grown robust rabbit, fed on bran with 1 ccm. of virulent broth-culture, held out for about $3\frac{1}{2}$ days before it died (from "chicken-cholera").

On the other hand, it was now and then, but comparatively seldom, observed that fresh wild rabbits (also one tame one), which had partaken of food contaminated with as much as 1 ccm.-2 ccm. of fresh broth-culture, did not at all succumb subsequently, and if so, not to "chicken-cholera."

In about half the number of instances I am inclined to ascribe the reason for these failures to the circumstance that the respective rabbits, although having been somewhat starved before, waited for hours before eating of the food (green leaves), and that, in consequence, the infectious matter on it was exposed to the drying effects of a summer temperature, disastrous to the microbes. In this way, it may be urged, the virulence might have been lost altogether, or if a certain portion of active material was preserved, it was perhaps not sufficient to infect by way of the digestive organs.

Such an explanation, however, cannot be adduced in favour of four other cases (three wild rabbits fed on 1 ccm. of broth-culture; one tame rabbit fed on about $1\frac{1}{2}$ ccm.); nor can it be maintained that in those cases the quality of the material employed was

to be blamed for the negative issue, because other rabbits treated under exactly the same conditions promptly perished. Be that as it may. That such rabbits as resisted in the first instance, were not, or had not become protected against the disease—except the tame rabbit mentioned, the history of which is given on pp. 522-525—was proved by their succumbing to it when they were in a satisfactory manner fed, in the second instance, on 2 ccm. of broth-culture, some time afterwards (one rabbit, however, died more than $2\frac{1}{2}$ days after the first feeding, from some indifferent cause, and another was lost sight of, before the ultimate proof of its susceptibility or otherwise could be given).

It has already been pointed out that the disease set up by the chicken-cholera microbes in rabbits, both by inoculation and feeding, mostly takes a rapid course. Although the term "chicken-cholera" for the disease caused by the microbes in rabbits, is inappropriate, I have made use of it for the sake of brevity and a better understanding.

The incubation occupies most of the time, the symptoms, or the actual disease being only of short duration. Death occurs under clonic cramps, and dyspnœa. Observations about the body-temperature during the disease, and some data regarding the breathing at the end of it, will be found in connection with experiments on the transmission of the disease from rabbit to rabbit (see pp. 554, 555, Table III.).

At the *post-mortem* examination one finds the following noticeable features :—The heart is filled with blood. The lungs are discoloured ; they are very voluminous owing to an emphysematic œdema involving their entire substance (on cutting through with a pair of forceps, or a scalpel, a crepitant sound is heard, and froth left on the blades of the instruments). Their surface presented a shining, mottled or tesselated appearance, due to ecchymoses or hæmorrhages in the lungs.

Pleura and peritoneum were mostly inflamed. The pleural, pericardial, and peritoneal cavities filled, as a rule, with serous exudations.

The spleen did not present any characteristic appearance.

The intestines were more or less hyperæmic, but of all the dozens of cases examined—rabbits fed as well as inoculated—I have only once met with a severe inflammation of the small intestine, the contents of which consisted of *blood-stained* liquid slimy masses. This was in the case of a vigorous, full-grown rabbit, fed at noon, February 19th, 1889, on cabbage-leaves and 2 ccm. of fresh broth-culture, and found dead at 7 a.m. next day.*

The rectum, or lowest portion of the large intestine, showed nearly always normal-looking fæcal masses, balled as usual; it was only rarely that its contents were not in the shape of isolated, well-formed "spheroids," but in that of soft, more or less coherent, greenish material.

Very frequently the rabbits, soon after death, had the nostrils covered with froth, which was stained with blood once. On the other hand, when the dead rabbits were kept for some time, in warm weather, undisturbed, in an open place, a blood-stained discharge from the nostrils was noticed repeatedly.

In conclusion, I may add that in the cases of inoculation, the seat of inoculation showed, as a rule, a slightly hæmorrhagic and gelatinous œdema. (One remarkable exception is that of a rabbit already mentioned above, as living two days after inoculation (see also p. 552); other noticeable exceptions are given by rabbits previously treated (see pp. 523-525, 529, 530).

The absence, as a rule, of hæmorrhagic exudations into the intestinal canal, and, as a standard, of diarrhœa proper, in rabbits treated with chicken-cholera bacteria, either by means of feeding or of inoculation, forms a fundamental difference from what we

* Hæmorrhage of a different character took place in a pregnant doe, which formed one of two fresh rabbits placed in a wire-bottomed hutch with one which had been given 2½ ccm. of virulent broth-culture (conf. p. 534). The doe, which was to all appearances in the end of the first, or the beginning of the second week of gestation, died from "chicken-cholera" by "contact," in less than 64 hours after being put in the hutch. Part of the fœtuses were found to have been aborted under *severe hæmorrhage*.

are accustomed to find in poultry affected with chicken-cholera. "Chicken-cholera" of rabbits has the character of a pure, most acute septicæmia, and is not a septicæmia in combination with "typhoid," as in poultry. In the judgment of the results obtained from certain experiments, we shall have to take this fact into consideration.†

History of Experiments on a Tame Rabbit.

1888.

(a) August 16th, 11.30 a.m.

A tame rabbit (♂, full-grown, long-haired albino, of the Angora type; not treated so far with "chicken-cholera" or anything similar) was *fed*, together with another tame, long-haired black rabbit (in one box), on cabbage-leaves infected with 3 ccm. of a virulent broth-culture of the chicken-cholera microbes. Both began to eat at once and had quickly finished eating the portion.

Results:

The black specimen was found dead (from "chicken-cholera") at 8.45 p.m., August 18th (again mentioned under "Experiments on Hares," p. 569).

† I regret not to have had at my disposal active cultures of the microbes of Koch's rabbit-septicæmia. Dr. Fischer, of Sydney, handed me on the 7th July, 1888, Agar-Agar-cultures of these microbes, which he had brought from Koch's Laboratory when in Berlin some time before. On examination, however, they were found to have lost their vitality.

I should have liked to study such bacteria side by side with the bacteria of chicken-cholera. The difference, so far made out between the two, is one of degree rather than of kind.

In the blood of the rabbits (as well as in other animals which in my experiments died of chicken-cholera, see below) the bacteria, in properly stained cover-glass preparations, appeared in the shape of the well-known rods which showed only the ends deeply coloured, while a middle portion presented itself as a colourless spot, with delicate, coloured lines laterally.

In liver-blood of rabbits dead of "chicken-cholera," I repeatedly observed that among the large numbers of typical microbes, there occurred, here and there, rather anomalous forms, which had about the same outlines, and behaved towards methylene-blue in the same way as those typical forms, but which were very considerably larger. Their length was up to 0·004 mm. (cover-glass preparations), their width about a third of length.

The other white specimen was still alive, August 20th; meanwhile it behaved in quite a normal manner.

(b) August 20th, 9.30 a.m.

It was given cabbage-leaves with 3 ccm. of virulent broth-culture of the microbes of the third generation. It began to eat at once, and had soon finished.

Result:

It was still alive, August 25th, not showing any symptoms of illness all the time. Two control-rabbits (wild), of which one (full-grown) received the same quantity of virulent material as the tame one, namely 3 ccm., and the other (half-grown) only half as much, namely $1\frac{1}{2}$ ccm., were both found dead at 8 a.m., August 21st. *P.M.* in each case, *Positive.*

(c) August 25th, 10 30 a.m.

It was given $4\frac{1}{2}$ ccm. of an active broth-culture of the microbe of the third generation, along with cabbage-leaves. It was not slow in doing away with the portion of infected food given.

Result:

It remained unaffected by this treatment, whereas a vigorous wild Tasmanian rabbit, taken as control, was observed to die at 8.30 a.m., August 26th, or about 22 hours after feeding. The cause of death, "chicken-cholera."

(d) September 1st, 4 p.m.

It was *inoculated*, subcutaneously at the left side of the belly, with $\frac{1}{8}$ ccm. (2 minims) of a virulent broth-culture of the microbes, obtained directly from the blood of a rabbit which died after feeding with those microbes. It outlived this operation, whereas a control rabbit (wild) was found dead (from the disease) at 8 a.m., September 2nd. At the seat of inoculation, however, in the case of the tame rabbit, was formed a large abscess, at first closed, but beginning to open five days after inoculation, thereby discharging a sticky, yellowish, inodorous pus. (A platinum-loop full of the latter was *inoculated* into a wild rabbit, at 12.45 p.m., September 10th. This animal was found dead at 7.30 a.m., September 13th, having perished from causes independent of "chicken-cholera").

The abscess healed slowly; the healing process was completed in the beginning of October.

(e) October 10th, noon.

The rabbit received injected, on a corresponding spot on the right side of the belly, $\frac{1}{8}$ ccm. (2 minims) of fresh heart-blood from a rabbit out of those recorded in Table III., *Series X.*, No. 19). As control-animals may be taken, on the one hand, the two rabbits from *Series XI.*,

Table III., which died in 14h. 21m., and between 7h. 29m. and 9h. 29m., after inoculation, respectively, (quantity for inoculation only 1-40th ccm.); on the other hand, the fowl and pigeon from *Series III.*, Table IV., which died between 20h. 15m. and 21h. 40m., and between 14h. 15m. and 20h., after inoculation, respectively, (quantity for inoculation only 1-40th ccm.). The tame rabbit did not become seriously indisposed. It reacted again through the formation of an abscess at the point of injection, and a higher body-temperature for some time after the operation.

Remarks on Body Temperature, &c. :—

October 10th—At time of inoculation (noon) ... 40·4° C.
,, ,, 5.55 p.m. ... 41·0°
,, ,, 10.15 p.m. ... 41·2°
October 11th— 11 a.m. ... 40·47°
,, ,, 3.30 p.m. ... 40·2°
,, ,, 10.10 p.m. ... 40·86°

On the morning of this day this rabbit's appetite was not so keen as usual. The seat of inoculation inflamed.

October 12th— at 3.15 p.m. ... 40·6°

October 13th— 1.15 p.m. ... 40·05°

October 18th—A distinct closed abscess, elastic to the touch, pink at surface, and of pear-shape.

October 19th—Abscess still closed, measuring 30 mm. in length (from apex to base), 23 mm. across the widest part, raised about 12 mm. above the level of the adjoining portions of the skin of the belly.

October 22nd—Abscess still closed, but apparently smaller.

October 26th—Abscess apparently discharging pus through a small hole. By pressing, pus of a thick, tenacious, and inodorous nature was obtained. The microscopical examination of samples of this pus (cover-glass preparations coloured with methylene-blue solution) did not disclose any chicken-cholera bacteria.

November—Traces of abscess disappearing.

1889.

(f) May 9th, 12.45 p.m.

The tame rabbit (which, I may mention here, was from the first to the last treatment, and afterwards, kept in a large enclosed place) was again treated, after an interval of *seven* months. This time I injected 1-24th ccm. (⅔ minim) of fresh heart-blood (from a rabbit dead of "chicken-cholera" after inoculation) under the skin at the back, between the shoulder-blades. Another vigorous wild rabbit was subjected to the same

treatment. The latter was found dead at 9.50 p.m., same day, it having died between 9 p.m. and that time (*i.e.*, between 8 and 9 hours after inoculation). (Result of *P.M.* examination, "chicken-cholera.")

The tame rabbit appeared somewhat indisposed on the evening of the same day, and on the morning of the following day. After that time it behaved as lively as usual, ready to eat any food given to it. But this time again an abscess developed itself at the place of inoculation, without having, however, any fatal effects on its bearer. The abscess discharging again copious quantities of pus, had almost completely healed up in the middle of June ; on the other hand, under the skin to the left of the seat of the abscess, a hard, freely movable nodule of about nutmeg- shape and -size was noticed. When seen, June 29th, the wound had completely healed up and the nodule disappeared.

Other Experiments on Rabbits regarding Immunisation.

It is known that Pasteur succeeded in conferring immunity against infection by virulent chicken-cholera bacteria, on fowls which had previously been inoculated with liquids obtained by filtering virulent broth-cultures of those bacteria through a Pasteur-Chamberland filter. The bacteria being thus eliminated, the effect produced by the filtrate must be ascribed to soluble substances resulting from the growth of the bacteria in the culture-fluid.

The results of a few similar experiments on rabbits are published by Prof. P. Foà and Dr. A. Bonome, in Turin.* By repeated injections of filtered broth-cultures of the chicken-cholera microbes into a rabbit, and subsequently of active culture, the death of the animal from chicken-cholera occurred at a considerably later date than that of a control-rabbit. By injecting successively larger doses of filtrate, and more frequently, a rabbit was rendered altogether insusceptible to a subsequent inoculation with such active microbes as were able to kill a fresh rabbit after a certain time.

* Ueber Schutzimpfungen. *Zeitschrift für Hygiene*, Band V., Heft 3, 1889, p. 423.

In the following I record a number of experiments which were undertaken with a view to ascertaining, whether it was possible to protect rabbits from the effects of virulent chicken-cholera bacteria, by administering to them such liquids in which the virulent microbes had propagated, but were afterwards killed by moderate heat. A preliminary experiment had shown me that, by immersing ordinary thin-glassed test-tubes containing fresh broth-cultures of the microbes, in water kept at 60°C. (140°F.), samples of the contents derived after 15, 30, 45, 60 minutes, were proved to be completely sterile in each case. Such sterilised cultures I employed of two kinds. The one description of culture-liquid was plain rabbit-broth, of slightly alkaline reaction; the other rabbit-broth, to which had been added 1 p.c. peptone and 0·5 p.c. salt; reaction the same. The cultures to be sterilised were left in the water-bath of the above temperature for 30 minutes.

I selected ten full-grown, well-conditioned wild rabbits, having been kept on the Island among others, which served me for control-experiments, for about three months. They had so far not been experimented upon, except that they had for some time previously been in an enclosure separated, by means of a double fence of rabbit-netting with fully a yard of space between, from another portion of the same enclosure in which wild rabbits were allowed to die of "chicken-cholera," and the dead bodies not removed until some time afterwards. This was, as may be seen later on, for the sake of testing the value of the disease with regard to its possible spread from infected to healthy rabbits under certain conditions.

The ten rabbits were placed separately in clean, spacious, sheltered hutches. I first intended to administer the different quantities of sterilised cultures directly *per os;* on finding, however, (by trial on an indifferent rabbit) this procedure not safe enough, I gave them to the rabbits in a small portion of bran, of which they were very fond. Bran was also used in these experiments when virulent broth-cultures were fed. To induce the rabbits, the control-rabbits included, to eat the portions given to them at once,

they did no receive any food, except water, on the morning of the day when (soon after noon) they were to eat the specially prepared food (conf. Footnote, p. 533). The result was quite satisfactory.

In order to avoid repetitions, I will mention here that all the broth-cultures, both those to be sterilised and those to be used in their active state—in the latter case plain rabbit-broth only was the nourishing medium—had been obtained from fresh heart-blood of rabbits, inoculated for that purpose with virulent broth-culture of the microbes. Such blood was tranferred in small quantities by means of a platinum-loop into the culture-tubes which had been warmed before in the water-bath, so that the broth contained in them showed already a temperature of some thirty degrees Centigr. They were then placed in a thermostat, where they remained for about 24 hours at a temperature close on 37·7°C., roughly speaking, between 37·5°C. and 38°C. They were then used immediately afterwards.

The plan of feeding the ten rabbits on sterilised cultures was as follows :—Two of them were to receive three successively increased portions at certain intervals, the next two one more than the first, the third one more than the second, and so on.

Section I.

1889.

Two rabbits were fed *three* times on steadily increased quantities of sterilised culture in peptonised broth (for one) and plain broth (for the other), as follows :—2 ccm., April 16th ; 4 ccm., April 17th ; 6 ccm., April 19th.

On April 21st, at about 1 p.m., up to which time the two rabbits appeared perfectly normal, they, as well as a vigorous control-rabbit, were given each 1 ccm. of active broth-culture in some bran. The control-rabbit died between 6.30 a.m. and 7.45 a.m., April 23rd, of "chicken-cholera." One of the principal rabbits, namely that previously fed on sterilised peptonised broth-culture, was seen to die at about 7 a.m., April 24th, of typical "chicken-cholera," as the subsequent examination proved.

The other rabbit which had been treated previously with sterilised plain broth-culture, being still alive April 27th, was given on that day, 2 ccm. of active broth-culture.*

It was still lively May 4th, when it was again fed, at about 1 p.m., this time on 3 ccm. of virulent culture. While another fresh, very robust rabbit, fed on 2 ccm. only, succumbed at 3 p.m., May 5th, to the disease, the former survived.

On May 10th, at about 2 p.m., it received 4 ccm. of active broth-culture; the same quantity was given to a control-animal which, however, had not finished eating it until 4 p.m. same day. The latter died between 2.15 p.m. and 2.35 p.m., May 11th, of "chicken-cholera;" the principal rabbit survived.

On May 15th, at about 2 p.m., this rabbit, and a control-rabbit, were fed on 6 ccm. of virulent broth-culture. The latter perished of "chicken-cholera" at 12.40 p.m., May 16th, *i.e.*, about 22½ hours afterwards. Neither did the former withstand this time; it died at 10.50 p.m., May 16th, *i.e.*, about 33 hours afterwards. On *post-mortem* examination, the carcass was found to be very stiff as usual; typical bacteria in preparations of the blood; but, with the exception of a pleuritis and a slight emphysema of the right lung, the organs looked normal. (Weight of the rabbit, 1490 grammes).

Section II.

1889.

Two rabbits were fed *four* successive times on the following quantities of sterilised culture in peptonised broth and plain broth, respectively: 2 ccm., April 16th; 4 ccm., April 17th; 6 ccm., April 19th; 10ccm., April 21st.

On April 23rd, at 1.15 p.m., they, as well as a control-rabbit, were given 1 ccm. of active broth-culture. The latter died at 2.30 p.m., April 24th, of "chicken-cholera;" of the two former, one previously treated with sterilised plain broth-culture died about a quarter of an hour later, also of "chicken-cholera."

The other rabbit being still alive April 30th—it never exhibited any suspicious symptoms—was fed again on that date, at 2 p.m., on 2 ccm. of virulent broth-culture. It was found dead at 6.30 a.m., May 2nd, whereas another fresh rabbit fed at the same time, along with others, on only 1 ccm. of the same culture, was found dead at about 7 a.m., May 1st. Both succumbed to typical "chicken-cholera."

* As will be seen further below, the two rabbits of *Section IV.* and a control-rabbit were fed, the same day, on 1 ccm. of the same culture for each rabbit. Although this control-rabbit survived this time, and only one of the former died of the disease 22 hours after being fed, the virulence of the employed culture cannot be doubted.

Section III.

1889.

Two rabbits were fed *five* successive times on sterilised cultures either in peptonised, or in plain broth, as follows: 2 ccm., April 16th; 4 ccm., April 17th; 6 ccm., April 19th; 10 ccm., April 21st; 15 ccm., April 23rd.

On April 25th, at 1.15 p.m., these two rabbits, as well as a control-rabbit, were given 1 ccm. each of virulent broth-culture. One of the two first mentioned, namely that previously fed on sterilised peptonised cultures, died between 1 p.m. and 2 p.m., April 26th; the control-rabbit succumbed considerably later, it being found dead at 6.40 a.m., 29th April, *i.e.*, roughly speaking, after $3\frac{1}{2}$ days. The cause of death each time was typical "chicken-cholera."

The rabbit previously treated with sterilised plain broth-culture, being still alive on April 30th, was fed at about 2 p.m. that day, on 2 ccm. of active broth-culture. It survived again, without offering any sign of a change in its behaviour, while a control-rabbit, fed on 1 ccm. only, along with others on the same date (see *Section V.*, mentioned also in *Section II.*), was found dead (from "chicken-cholera") at about 7 a.m., May 1st.

On May 4th, at 1 p.m., the above rabbit was given 3 ccm. of active broth-culture. A very robust control-animal which received 2 ccm. of the same culture (as also did two other rabbits treated before), died at 3 p.m., May 5th, of typical "chicken-cholera." The principal rabbit remained alive and well.

On May 10th, at about 2 p.m., 4 ccm. of virulent broth-culture were given to it. It survived again without, apparently, the least inconvenience. A control-rabbit, as already mentioned in connection with the rabbit under *Section I.*, of the same date, succumbed about 24 hours afterwards.

On May 15th, at about 2 p.m., the rabbit received 6 ccm. of active broth-culture. It withstood also this time, without showing any abnormal symptoms. A control-rabbit, as already mentioned under *Section I.*, died about $22\frac{1}{2}$ hours after feeding.

On May 21st, six days after the last feeding on 6 ccm. of culture, the rabbit was *inoculated* with a small quantity of heart-blood, derived from a rabbit which had perished about 6 hours since, of typical "chicken-cholera" consequent on inoculation with virulent broth-culture. The quantity, namely 1-48th ccm. ($\frac{1}{3}$ minim) was injected by means of a pointed glass-tube, under the skin at a spot on the belly. Another fresh rabbit, of the same sex (♂) and about the same size, served for control-inoculation. This control-rabbit died at 1.10 a.m., May 22nd, or 13 hours afterwards; the autopsy as well as the result of the microscopical examination of cover-glass preparations of blood, secured the diagnosis—"chicken-cholera."

The principal rabbit remained alive for good, but exhibited the following symptoms :—At the place of inoculation there was formed a rather large abscess which began to discharge pus for some time, and through which a necrotised portion of muscle and skin was eliminated, similar to the process which may be observed in fowls. The rabbit, which had always a good appetite, became somewhat thinner; when seen again on June 15th, it was as well-conditioned as before the experiment; the wound was then not quite healed up. When seen on June 29th, the healing was perfect.

Section IV.

1889.

Two rabbits were fed *six* successive times on sterilised cultures in peptonised broth or in plain broth, respectively, namely: the first five times exactly as under *Section III.*, and on the same dates; the sixth time on 22 ccm., April 25th.

On April 27th, at 1 p.m., each of them, and a control-rabbit, were given 1 ccm. of virulent broth-culture. The rabbit previously fed on sterilised peptonised cultures, died from typical "chicken-cholera" at 11 a.m., April 28th, that is 22 hours after feeding. The other rabbit, as well as the control-animal, did not succumb this time.

On May 4th, at about 1 p.m., both received 2 ccm. of active broth-culture each. A control-rabbit died at 3 p.m., May 5th (the same already mentioned under *Sections I.* and *III.*, May 4th); the rabbit previously treated with sterilised plain broth-cultures perished between 8.45 a.m. and 9.30 a.m., May 6th, that is about 44 hours after feeding, whereas the original control-rabbit was found dead at about 6.30 a.m., May 7th, it having died between 9 p.m., May 6th, and that time.

Section V.

1889.

Two rabbits were fed *seven* successive times, of which the first six were as in *Series IV.*, and the seventh time was on April 27th, when 45 ccm. of sterilised culture, either peptonised or plain, were given.

On April 30th, at 2 p.m., each of them, as well as a control-rabbit, received 1 ccm. of virulent culture. The last-mentioned rabbit was found dead at about 7 a.m., May 1st (as already notified under *Sections II.* and *III.*). The rabbit formerly treated with sterilised peptonised cultures died between 10 a.m. and 11.15 a.m., May 3rd, or somewhat less than 3 days after feeding; cause of death, typical "chicken-cholera." The other rabbit, treated with sterilised plain broth-culture, did not become affected.

On May 4th, at about 1 p.m., it was fed on 2 ccm. of active culture. It died at 4.30 p.m., May 5th. At the *post-mortem* examination everything

was found as in ordinary rabbits dead from the disease. A control-rabbit died 1½ hours before, at 3 p.m., that day (as already mentioned under *Sections I., III.* and *IV.*).

According to the results thus obtained in the foregoing experiments, which are not numerous and not varied enough to admit of any definite conclusions to be drawn, the possibility of the protective power, on rabbits, of sterilised broth-cultures introduced successively into the digestive canal, against a subsequent infection by active cultures, can hardly be denied. We see that a subsequent feeding on 1ccm. of virulent culture had in several cases not the slightest effect on previously treated rabbits, while control-rabbits succumbed, with one exception (1 ccm.). Continued feedings up to 6 ccm. (two cases) of active material caused the death of all rabbits except one, out of *Section III.* This rabbit survived even inoculation, of which another fresh rabbit perished quickly.*

Cultures in peptonised rabbit-broth, and sterilised, proved themselves, against my expectation, inferior to such made in plain rabbit-broth, as regards their protective influence on rabbits.

Is "Chicken-cholera" a Contagious Disease among Rabbits?

The question as to whether, or to what degree, rabbits suffering or dead from "chicken-cholera," are able to communicate the fatal disease to other healthy rabbits with which they are associated, was one that engaged my attention for a considerable time.

Experiments by Pasteur and his Representatives.

Pasteur states that fresh rabbits placed with others which have partaken of food contaminated by virulent chicken-cholera microbes, die in large numbers.†

* If possible, and unless the rabbit should die from some cause or other, I intend to try another inoculation several months after the first.

† Sur la destruction des lapins en Australie et dans la Nouvelle-Zélande *Annales de l'Institut Pasteur*, 2me année, 1888, p. 6.

Five tame rabbits, in one box, were fed on infected food, and 6 hours later three fresh ones (not contaminated) were introduced into the same box. Apart from the five former, one of the three latter succumbed to "chicken-cholera." *

In another experiment, four tame rabbits received microbe-contaminated food, and 7 hours later when all the food had disappeared since several hours, four new rabbits were penned up in the same box with the four first ones. The carcasses of these four infected rabbits, which died within 23 hours, were left in the box. All the four additional rabbits were dead from "chicken-cholera" within six days from the beginning of the experiment.†

An experiment on a large scale‡ made by Loir, at Pasteur's instigation, on Mme. Pommery's Estate, at Reims, on the rabbits in an enclosure of eight hectares (about twenty acres), resulted in killing off the whole number of rabbits there, which were estimated at more than a thousand. According to the evidence given before the Rabbit Commission in Sydney by Pasteur's representatives, it was considered as probable that the mortality among those rabbits was partly due to the transmission of the "chicken-cholera" virus from rabbit to rabbit. In my opinion, this wholesale mortality can satisfactorily be explained without taking to "contagion."

Lastly, I adduce the experiment of demonstration performed by Pasteur's delegates at Rodd Island (Sydney). *Five* wild rabbits, fed in one cage on cabbage-leaves sprinkled with 5 ccm. of a virulent broth-culture, were soon afterwards placed among *twenty* fresh rabbits (also wild) in a four-sided wooden enclosure of only one square metre area (about 3′ 3½″ square), in a stable-stall. The observation extended to a period of ten days. Within this period *eleven* rabbits in all died, among these, *three* (specially marked) of the *five* which had been given infected food, while *one* of the latter survived. The fate of the *fifth* of the originally infected rabbits could not be ascertained, because, inadvertently, it had not been marked. Accordingly, either *seven* or *eight* of the *twenty* uninfected rabbits died. All the dead rabbits were left in the enclosure until the demonstration was concluded, with the exception of *three not marked* ones which were removed during the experiment for examination (among these, *one* infected one might or might not have been, to judge from what has been stated above). In consequence of this examination, the diagnosis "chicken-cholera" could be given in each case. In order to fully decide whether the other *unmarked* rabbits (five) also perished of "chicken-cholera" or not, a *post-mortem* examination would have been necessary; this, however, was not made.

* loc. cit., pp. 4, 5.
† loc. cit., p. 5.
‡ loc. cit., pp. 7, 8.

Own Experiments.

In my official reports full details (with illustrations) are furnished about the experiments undertaken by me with a view to obtaining what information was considered by the Commission as worth having. Here it may suffice to give a *résumé* of their arrangements and their results.

Generally speaking, such experiments were conducted :—

A. On infected and uninfected rabbits mixed together

I. In wooden hutches, either with wooden bottoms or wire-netting bottoms.

II. In enclosures containing artificial burrows.

B. On intact rabbits placed

III. In boxes or hutches, in which rabbits had died from "chicken-cholera."

Ad I.

(a) On September 3rd, 1888, ten full-grown rabbits were fed,* in separate cages, on cabbage-leaves to which was added a small quantity of virulent broth-culture of the chicken-cholera microbes.† This quantity was 2½ ccm. each for eight of the ten, 1 ccm. each for the two remaining ones.‡ Soon afterwards, when all the food had disappeared except in one cage, where only about half was eaten, the *ten* rabbits were placed, in the proportion of one to two, with *twenty* uninfected rabbits, of which six were only half-grown, in *eight* hutches, as follows :—*six* hutches (measuring in the clear inside 23″ × 18″ × 18″ in

* Whenever, during the course of my experiments, rabbits were to be fed on "chicken-cholera"-contaminated food, I adopted the precaution of starving them to some slight extent beforehand, in order to induce them to eat the infected meal given to them more readily. In spite of this arrangement it sometimes happened that the one rabbit or another was slow in touching the food, or finishing it up. Wild rabbits, when suddenly penned up in hutches, are naturally very shy and suspicious at first.

† In order to be sure on this and all other occasions, when green leaves were used, that the infective material adhered firmly to the food, and that the danger of the broth becoming detached or perhaps lost, while the rabbits were eating, be avoided as much as possible, each portion was prepared on a soup-plate, where the culture, which was sprinkled out of a fine-pointed measured glass-tube, was placed between leaves or portions of such, and these repeatedly pressed down, and turned by aid of flat wooden sticks.

‡ The history of the culture employed is as follows :—Colony from virulent blood of a rabbit (fed on culture), 10/VIII. 1888 = I. generation ; stick-culture in 6 p.c. rabbit-broth-gelatine, 14/VIII. = II. generation ; stick-culture, 18/VIII. = III. generation ; rabbit-broth culture, 1/IX = IV. generation. The latter, when used September 3rd, had been since in thermostat at 33-35° C. for two days.

depth, height and width, respectively; three were wooden-bottomed, three wire-netting bottomed, the latter resting on sandy soil) were stocked with *three* rabbits each; *two* hutches (3′ 3½″ square, 2′ high; one wooden-bottomed, the other wire-netting bottomed, placed as before §) were stocked with *six* rabbits each; here, as well as there, always in the number of one infected to two uninfected specimens. The experiment lasted *seven* days. *Eight* of the ten infected rabbits promptly died from "chicken-cholera," as proved by the *post-mortem* examination—they were removed from their hutches soon after death—and by control-rabbits. All these had been fed on 2½ ccm. of culture. Of the remaining two, however, which had received only 1 ccm. culture, one died (after more than 2½ days) from some indifferent cause, and the other survived this time, while a control-rabbit (1 ccm.) succumbed to the disease. Of the *twenty* originally uninfected rabbits, *four* contracted "chicken-cholera," and died in consequence, in the smaller hutches, namely—*two* in one with bottom of rabbit-netting; *one* each in a wooden-bottomed and wire-netting bottomed hutch. I need hardly say that these *four* rabbits, which perished in from about two days and a half to four days seven hours after the beginning of the experiment, had been together with rabbits which, after feeding on 2½ ccm. culture, quickly succumbed, as mentioned above.

I have also to record the death of *ten* other (including five half-grown) rabbits out of the original twenty, within the seven days, but I was unable to trace, as cause, "chicken-cholera."

(b) On September 10th, 1888, *two* rabbits were fed on green barley and virulent culture (derived directly from the blood of one of the rabbits dead from "chicken-cholera" by "contact," in one of the hutches of the preceding experiment). *One* of them received 1 ccm.; the *other*, which was the surviving one from the former experiment after feeding of 1 ccm., was given 2 ccm. this time. They were placed in two of the smaller hutches (see above), one having a bottom of wood, the other one of rabbit-proof netting (as before), with one full-grown and one half-grown rabbit for each. The *two infected* rabbits died speedily from "chicken-cholera" (they were removed from their hutches soon after death); of the *four uninfected* rabbits, the two half-grown and one full-grown died within the first three days; the result of *post-mortem* examination was each time negative as regards "chicken-cholera." The other full-grown specimen was still alive after seven days.

§ All the eight hutches were placed in the large wire-gauze enclosure on the Island. Six of them (the small-sized) were so placed as to prevent the sun completely from shining into them; the inside of the two larger ones was only to a slight extent accessible to the sun.

NOTES on the *Temperature* (in the shade) taken during the course of the experiments. The temperatures are given in the centigrade scale.

September 3rd.	7 a.m. 7°	1.45 p.m. $16\frac{3}{4}$°	6 p.m. $13\frac{3}{4}$°	10.30 p.m. $10\frac{1}{2}$°	September 11th.	7.15 a.m. $10\frac{3}{4}$°	2 p.m. $12\frac{1}{2}$°	5 p.m. $11\frac{3}{4}$°	10.15 p.m. 12°
4th.	7.15 a.m. 10°	1.30 p.m. 18°	5 p.m. $14\frac{1}{2}$°	9.30 p.m. $10\frac{1}{4}$°	12th.	7.30 a.m. $9\frac{1}{2}$°	1.45 p.m. $16\frac{1}{4}$°	.....	Midnight. $14\frac{1}{2}$°
5th.	7 a.m. $7\frac{1}{4}$°	1 p.m. 20°	5.30 p.m. 16°	10.15 p.m. $12\frac{1}{4}$°	13th.	7.30 a.m. 9°	1 p.m. $21\frac{3}{4}$°	5 p.m. $16\frac{3}{4}$°	10 p.m. $12\frac{3}{4}$°
6th.	7.15 a.m. $8\frac{3}{4}$°	1.30 p.m. 24°	5.15 p.m. 18°	10.45 p.m. $14\frac{1}{4}$°	14th.	7.30 a.m. $10\frac{3}{4}$°	1.30 p.m. 21°	5 p.m. $16\frac{1}{2}$°	10.30 p.m. $9\frac{3}{4}$°
7th.	7.30 a.m. 12°	1.45 p.m. 15°	5 p.m. $14\frac{1}{4}$°	10.20 p.m. 10°	15th.	7.30 a.m. $9\frac{1}{4}$°	2 p.m. $19\frac{3}{4}$°	5 p.m. $16\frac{3}{4}$°	11 p.m. 13°
8th.	7.15 a.m. $8\frac{3}{4}$°	2 p.m. 16°	5.30 p.m. $15\frac{3}{4}$°	10 p.m. $9\frac{1}{2}$°	16th.	8 a.m. $12\frac{1}{4}$°	2 p.m. $21\frac{3}{4}$°	5.30 p.m. 19°	10.30 p.m. $15\frac{1}{4}$°
9th.	7.30 a.m. $9\frac{3}{4}$°	1.30 p.m. $14\frac{1}{2}$°	.....	11 p.m. 9°	17th.	7.30 a.m. $16\frac{1}{4}$°	1.30 p.m. $19\frac{1}{2}$°	5 p.m. $17\frac{3}{4}$°	.....
10th.	7.15 a.m. 8°	1.45 p.m. $16\frac{1}{2}$°	5.30 p.m. $14\frac{3}{4}$°	10.45 p.m. $12\frac{1}{2}$°					

During the above time (September 3rd-September 17th) it was generally calm and fine, only now and then it was damp or raining.

The result of these experiments is somewhat marred by the great mortality among the rabbits under observation, independent of "chicken-cholera." Nevertheless it shows that, similar to those obtained by Pasteur and his representatives, the possibility of a transmission of the disease from rabbit to rabbit, under conditions such as are described, is out of question.

Ad II.

(a) On August 28th, 1888, *eight* full-grown rabbits (also two others for immediate control) were fed on cabbage-leaves sprinkled with $2\frac{1}{2}$ ccm. of active broth-culture * for each. They were thereupon placed with *sixteen* uninfected rabbits (among which five half-grown) in special enclosures containing an artificial burrow each. These artificial burrows were constructed in the loose sandy soil which covers the surface of the large wire-netting and wire-gauze enclosure, and fenced in, at some distance, by rabbit-netting. They consisted in winding and branching trenches, as nearly as possible five inches deep and four and a half inches wide, covered with boards and soil so that they could easily be uncovered and inspected. They were provided with one entrance.†

Three rabbits (one infected, two uninfected) were turned into each of three small burrow-enclosures containing about 13′ 6″, 16′, 16′ 6″ of burrow, respectively; into another, with about 58 running feet of burrow, *six* rabbits (two infected, four uninfected) were let go; the last enclosure, in which were about 70 running feet of burrow, was stocked with *nine* rabbits (three infected, six uninfected). Within twenty-five minutes all twenty-four rabbits had found their way inside the burrows in their respective enclosures.

The *eight* infected rabbits (as well as the two others also fed on the same quantity of contaminated food) promptly died from "chicken-cholera," *six* outside, *two* inside the burrows. Their carcasses were left untouched on the spot, where found, for three full days.

Of the *sixteen* uninfected rabbits which, unless they died before, were to be left in the enclosures for seven days from the beginning, *six* in all (namely four full-grown, and two half-grown) died within this time. But not in one instance could the cause from which they died be identified as "chicken-cholera."

* The history of this culture is as follows:—Colony from virulent blood of a rabbit fed on culture, 10/VIII. '88=I. generation; gelatine-stick-culture, 14/VIII.=II. generation; broth-culture, 23/VIII.=III. generation; broth-culture, 26/VIII.=IV. generation. The latter remained, before use, in a thermostat at 35-37° C. for two days.

† They were constructed after data given by Mr. A. N. Pearson, of Melbourne, a member of the Royal Commission.

NOTES on the *Temperature*, both in air (shaded) and at the bottom of a separate small burrow or trench, without entrance (not used by the rabbits), in the following table called underground. Also notes on the *Weather* during the course of the experiments.

DATE.	TEMPERATURE (CENTIGR.).					WEATHER.
August 28th.	air (shaded) underground			4 p.m. 19°	7.45 p.m. $17\frac{1}{4}°$ 16°	Bright and calm all day.
29th.	air (shaded) underground	7 a.m. $13\frac{1}{4}°$ $15\frac{1}{4}°$	12.45 p.m. 16° $15\frac{1}{2}°$	5 p.m. $15\frac{1}{4}°$ $15\frac{3}{4}°$	11.15 p.m. $14\frac{1}{2}°$ $15\frac{1}{4}°$	Morning dull; strong southerly wind all day; night calm.
30th.	air (shaded) underground	7 a.m. $13\frac{1}{2}°$ $14\frac{3}{4}°$	3 p.m. 18° $15\frac{1}{2}°$	6 p.m. 16° $15\frac{1}{2}°$	11 p.m. $15\frac{1}{2}°$ 16°	Calm and fine all day.
31st.	air (shaded) underground	7 a.m. $13\frac{1}{2}°$ $15\frac{1}{4}°$	1.30 p.m. $24\frac{3}{4}°$ $16\frac{1}{4}°$	6 p.m. $20\frac{1}{2}°$ $16\frac{1}{2}°$	10.30 p.m. $18\frac{3}{4}°$ $16\frac{1}{2}°$	Calm and fine all day.
September 1st.	air (shaded) underground	7.15 a.m. 17° $15\frac{1}{2}°$	1 p.m. 14° $16\frac{1}{4}°$	5.15 p.m. $12\frac{3}{4}°$ 16°	10 p.m. 12° $15\frac{3}{4}°$	Morning dull, air damp, light showers; afternoon light rain; evening calm and fine.
2nd.	air (shaded) underground	8 a.m. $9\frac{3}{4}°$ 14°	2.30 p.m. $14\frac{3}{4}°$ $15\frac{3}{4}°$	5.15 p.m. $12\frac{1}{4}°$ $15\frac{1}{4}°$	10 p.m. $8\frac{1}{2}°$ $14\frac{3}{4}°$	Bright and calm.
3rd.	air (shaded) underground	7 a.m. 7° 14°	1.45 p.m. $16\frac{3}{4}°$ 15°	6 p.m. $13\frac{3}{4}°$ 15°	10.30 p.m. $10\frac{1}{2}°$ $14\frac{1}{2}°$	Bright, with light southerly breeze blowing.
4th.	air (shaded) underground	7.15 a.m. 10° $14\frac{3}{4}°$	1 p.m. 18° 15°			Calm; sky overcast; air damp.

(b) In this experiment the whole of the large enclosure, already referred to, was utilised. This enclosure, which measured 100 feet by 80 feet, contained artificial burrows, in all about 185 running feet.

On November 7th, 1888, *one hundred* rabbits, mostly full-grown and only a few half or not quite full-grown, were let loose in that enclosed place. The rabbits were, as the result later on showed, mostly in a poor condition. Shortly afterwards, *ten* rabbits which had been fed on cabbage-leaves sprinkled with 2 ccm. of active broth-culture for each rabbit, were placed with the former in the same enclosure. On November 14th, another batch of similarly infected rabbits, this time *six*, among which three Tasmanian ones, were introduced. Lastly, on November 22nd, a third batch of *six* infected rabbits,* also fed on 2 ccm. culture,† were let loose in the same enclosure. On November 29th the period of observation terminated.

Infected Rabbits.—Of the *twenty-two* infected rabbits thus turned loose among other uninfected ones, *twenty-one* succumbed, while the *twenty-second* survived. (It died, however, December 3rd, *P.M. negative.*) Of the *twenty-one*, *three* were removed from the enclosure shortly after they were found dead, and examined (one, each, of the first, second, and third batch). The result of the examination was in the first case *positive* (rabbit found dead inside burrow), in the second and third, *negative.* The other rabbits, *eighteen* in number, were not taken out of the enclosure until the conclusion of the experiment, November 29th. *Twelve* of these *eighteen* died outside, *six* inside the burrows.‡ The proof of those *eighteen* having died from "chicken-cholera" was furnished partly by control-experiments on other rabbits, partly by the appearance of the carcasses—which showed *rigor mortis* exceedingly well-marked, in contrast to other rabbits which perished from some indifferent causes (except, of course, any septicæmia similar in effect to chicken-cholera)—partly by the characteristic symptoms which some of the rabbits under consideration were observed to exhibit when dying, or some time before death. Lastly, the diagnosis was made sure by the positive results of the direct microscopical examination of cover-glass preparations of blood derived§

* The consignment of rabbits, of which these six formed part, had been received on the Island only the previous day.

† The cultures used in this experiment were derived directly from blood of rabbits dead from "chicken-cholera," and incubated at 39-40°C. for 24 hours before being used.

‡ The burrows, of course, were opened and examined from time to time.

§ This was done each time by means of a clean sterilised glass-tube, which had been drawn out in the flame into a fine end of some length. By pushing this fine end through a suitable spot at either the right or the left side of the thorax, from which spot the hair

from a certain number (eight) which were picked out at random each time, and of the full *post-mortem* examination of one which was found dead inside burrow at the conclusion of the experiment.

Uninfected Rabbits.—Of the *hundred* uninfected rabbits placed in the enclosure, November 7th, five died quickly up to the morning of November 8th, and were at once replaced by fresh ones. Thence to November 14th, when the second batch of six infected rabbits were let loose, not less than *fifty-two* had died.

From November 15th to November 22nd, when the third lot of *six* infected rabbits were let loose, *seventeen* had died.

From November 23rd to November 29th (conclusion of the experiment), *ten* had died.

Thus it will be seen that not less than *seventy-nine* out of the hundred died, partly inside, partly outside the burrows. One rabbit managed to escape, somehow or other, into the adjoining shed, about a week after the beginning of the experiment. It was used otherwise. So that not more than *twenty* of the *uninfected* rabbits were left over ultimately.

The carcasses of the *seventy-nine* rabbits did not in the least indicate that "chicken-cholera" was the cause of their death; nor did the symptoms which a number of rabbits were seen to show shortly before death, correspond with those characteristic in "chicken-cholera." The carcasses were all removed from the enclosure as soon as it was possible, and submitted to a careful examination. But not in one instance could the cause of death be diagnosed as "chicken-cholera." On the contrary, I had little doubt that the huge mortality encountered in this experiment among the hundred rabbits arose from the effects of the starvation which they had to undergo, to a certain extent, before they were sent to the Island from the then dry country round Hay, New South Wales. I should add that before and after the above experiment, a similar mortality was noticed among rabbits kept in stock, and that every attendance as regards feeding, sheltering, or the like, was given to the rabbits on the Island on all occasions.

(An appended table of temperatures and notes on weather prevailing during the term of the above experiment may be found at the end.)

had been removed previously, a sample of liver-substance was derived. The opening thus made into the body closed up again after the tube had been taken out, and in this way the body was not perceptibly disturbed.

(c) The foregoing experiment being unsatisfactory in its results, on account of the high mortality among the rabbits from causes other than "chicken-cholera," the Commission decided for another large experiment.

For this purpose, the main enclosure on the Island (see above) was divided into two nearly equal portions by means of a double fence of rabbit-netting, with a clear space of one yard (about 92 cm.) between. The one division, which may be called the disease-division, contained about 136 running feet of artificial burrow; these burrows were old ones, formerly used, but here and there altered. In the other division, henceforth called the control-division, there was a total of about 95 feet of artificial burrow; there were two of such burrows, one old one, somewhat changed, and another fresh made.

The arrangement was, to turn into each of these divisions *fifty* healthy rabbits, not fed on the chicken-cholera microbes; to add to the *fifty* in the disease-division *three* batches of *five* rabbits for each, which had been fed on fresh cabbage-leaves sprinkled with 2 ccm. of a virulent broth-culture of the microbe of chicken-cholera for each rabbit. The first batch was to be turned in at once, the second after a week, and the third after a fortnight; the experiment was to be completed after three weeks from the outset.

The experiment was begun on February 12th, 1889, and concluded March 5th, according to programme.

Although the whole enclosure had been used from November 7th to the 29th, 1888, for the carrying out of the experiment mentioned under (b), p. 538, I did not think it necessary to specially disinfect it, in view of the new experiment. From the end of November, after the former experiment, some twenty rabbits were left there till the 24th January, without anyone dying from "chicken-cholera." During the interval, sunshine and wind could act on, and must have proved disastrous to, any chicken-cholera microbes that might have been deposited there. Then again, one portion of the enclosure was, in the fresh experiment, reserved as control-division, stocked with a considerable number of rabbits; of these, I may just as well state beforehand, not a single one perished from "chicken-cholera."

The result of the experiment which was carried out as said above,* was as follows:—

*Throughout this experiment I employed broth-cultures which had been obtained directly from fresh heart-blood of rabbits, inoculated for that purpose with "chicken-cholera." The tubes containing the microbe-infected broth were placed in a thermostat kept at about 38° C., where they remained for about 24 hours before being used. A temperature of that degree appears to answer for the growth of the microbes better than any other.

Disease-Division.

The *fifteen* rabbits (all full-grown, well-conditioned specimens) which after being fed on 2 ccm. of virulent broth-culture for each, on cabbage-leaves, were let go in the disease-division—*five* on February 12th, *five* on February 19th, *five* on February 26th,—died promptly without exception; the majority of them must have died in less than 20 hours. *Nine* of the *fifteen* died outside, *five* inside the burrows,* and *one* half outside and half inside. Among the nine first mentioned is included one, which lay dead in a hollow covered over by a stone, and which was easily accessible.

With one exception, the carcasses of the rabbits remained on the spot where they were found lying, until the end of the experiment, without any microscopical examination of their blood being made. The exception referred to is a rabbit which, forming one of the last batch of five rabbits placed in the division, February 26th, was found dead the following day, outside burrows. It was, on examination, found to be much bruised on the left side of chest and belly, an occurrence which must have accelerated its death, as putrefaction of the organs had already set in when the examination took place, soon after the rabbit was found dead. However, the heart-blood clearly showed the presence of numerous bacteria of "chicken-cholera." An unusually vigorous buck, inoculated with a small quantity of such blood, succumbed to "chicken-cholera" somewhat less than twelve hours afterwards. On the following morning, the intact carcass of one of the control-rabbits (see below) which had died the previous evening, was put in the place of the one removed from the enclosure.

The *fifteen* rabbits lying scattered in the disease-division undoubtedly perished from "chicken-cholera." On the one hand, *fourteen* control-rabbits, which speedily died without a single exception, died from "chicken-cholera," as unmistakably shown by the results of careful examinations. On the other hand, the appearance of the carcasses, and the symptoms which some of the rabbits were observed to exhibit when dying, corresponded with what occurs in "chicken-cholera" rabbits.

Of the *fifty uninfected* (*i.e.*, intact) rabbits, let loose in the disease-division at the beginning of the experiment, *four* died from "chicken-

* The burrows were, of course, opened from time to time; in all nine times.

cholera," all inside burrows, whereas *thirty-two* perished from causes which had nothing in common with that disease.*

The way in which these thirty-six rabbits, which died out of the fifty, were examined, in order to see whether chicken-cholera bacteria had found their way into them or not, was not the same each time.

Twenty-two were at once subjected to a full examination ; for that purpose they were taken out of the enclosure soon after their death. Besides noting the condition of the organs, a microscopical examination of blood was made. In sixteen cases liver-blood, in two cases liver- and heart-blood, in two cases heart-blood only was examined ; in the latter two instances the liver being unsuitable. From two rabbits found dead inside a burrow, February 28th, and being in an advanced state of decomposition, a sample each of coagulated heart-blood was inoculated into a medium-sized rabbit. Of the twenty-two rabbits thus examined, only *one* (found inside burrow, February 16th) was proved to have taken "chicken-cholera," while in the others neither the autopsy, nor the microscopical examination of blood, warranted the same verdict.

From the remaining fourteen dead rabbits, while they were lying about, some liver-substance was taken (in the manner described previously), of which cover-glass preparations were made for microscopical examination. *Three* times a *positive* result was obtained, inasmuch as the typical bacteria of chicken-cholera, and only these, were present in large numbers. The three respective rabbits, which also by their outward appearance indicated death from "chicken-cholera," were left where they died, until the close of the experiment. A subsequent *post-mortem* examination (including microscopic examination of blood, seven times) of the eleven remaining rabbits, in samples of liver of which the microbes of chicken-cholera had not been found, confirmed the negative result arrived at previously.

To return once more to the *four* originally *uninfected* rabbits which subsequently succumbed to "chicken-cholera," I am confident that the germs of this disease could not have been supplied to those four but by intentionally infected rabbits placed in the disease-division. Not only was the greatest care taken in eliminating any possibility of carrying infectious material among the rabbits, through food or

* Of these thirty-two died :—within the first week (12th-19th February), four ; within the second (up to the 26th February), nineteen, of which six found dead on one day (February 23rd), and nine on another (February 25th); within the third (last) week (up to 5th March), nine.

through the necessary inspections, but also, as stated, a control-experiment on fifty rabbits in an adjacent enclosure was made, with the result that not a single death from "chicken-cholera" occurred there.

Two of these *four* rabbits were found dead (inside burrows; carcasses still well preserved) on the 16th February (*i.e.*, somewhat less than four days after the first batch of five infected rabbits was turned loose); the third was found dead (inside burrow; carcass still fresh) two days afterwards, on the 18th; the fourth (inside burrow; carcass still pretty fresh) on the 23rd (*i.e.*, somewhat less than four days after the second lot of five infected rabbits was let loose). The probability, therefore, is that all *four* rabbits became infected *after* the death of specially infected rabbits placed with them. The answer to the question, in what particular way this infection took place, is open to conjecture. Considering that the evacuations of normal rabbits, dead of "chicken-cholera" after either feeding or inoculation, do not, as a rule, exhibit anything abnormal in their appearance; considering, also, that within the short time which it took, in the case of the fifteen rabbits, from the time of infection until death, fæces originating from the infected meals could hardly have been excreted; and lastly, in view of the negative results of a few direct experiments made by me (see pp. 546-548), it is far from being proved that the excrements (or the urine) of the rabbits which died in the disease-division from "chicken-cholera" were or must have been the vehicles of infection. On the other hand, it was frequently noticed that from the nostrils of carcasses of infected rabbits lying undisturbed, several days after the death of the animals, a blood-stained liquid exuded. Here and there it was noticed that the maggots of a small fly, and the latter itself, also ants, were at work about the carcasses. All that may have yielded the means for transmitting the virus.

Control-Division.

The number of fifty intact rabbits to be placed in this division at the beginning of the experiment, was at that date short of twelve; two died in the enclosure a few hours after being put there. When, two days afterwards, a fresh supply of rabbits came to hand, fourteen of them were turned in, in order to make up for the number missing.

At the conclusion of the experiment only *twenty-one* live rabbits were left over, twenty-nine having died, partly inside, partly outside the

burrows,* during the time. In every instance it was proved that death was not owing to "chicken-cholera."

This unfortunate mortality in both the one and the other division, from causes different from "chicken-cholera," was, in my opinion, favoured to a large extent by the extremely oppressive atmosphere and the excessive heat experienced now and then during the course of the experiment.† In the appended Table II. may be seen records of temperature and general remarks on the weather for that period. In quite a number of rabbits the liver and the intestines were diseased, such an appearance resembling that noticed in wild rabbits which were partly starved, or were feeding on unsuitable food. In other rabbits, again, the lungs were pneumonic. So that, after all, it is to be regretted that in this second large experiment, the rabbits used were not all of them in a healthy condition either.

Ad III.

In five single experiments fresh rabbits were placed in boxes or hutches in which rabbits had died from "chicken-cholera"; these rabbits, however, were removed shortly after being found dead. The result was in each instance a negative one, inasmuch as a transmission of the disease, in those cases, was not observed.

1888.

(a) August 29th, 9.30 a.m.

A rabbit was placed in a box, ‡ in which two rabbits, *inoculated* with a portion of virulent broth-culture, were found dead, one at 7.45 p.m., August 28th, the other at 7 a.m., August 29th. In the box there

---

* The burrows in the control-division were always opened on the same days, on which those in the disease-division were examined.

† Most of them (twenty-three) died within eleven days from the beginning of the experiment; of these, six were dead on one day (Feb. 14th), and nine on another day (Feb. 23rd).

‡ The boxes or hutches alluded to in these experiments, were placed in the large enclosure on the Island in such a way that their insides were almost completely sheltered from the rays of the sun. For temperatures and weather during the experiments noted here, see p. 537.

was a considerable quantity of normal-looking fæces, and a large portion of the food given to the two inoculated rabbits on the evening of August 28th.

Result:

The rabbit was still alive, September 6th, when it was removed from its box.

(b) August 29th, 9.30 a.m.

A half-grown rabbit was placed in a hutch in which a rabbit, *fed* the day before on cabbage-leaves with 2½ ccm. of virulent broth-culture, was found dead (from "chicken-cholera") at 7 a.m., August 29th. The hutch contained a small quantity of normal-looking fæces; part of the bottom was damp with urine. The portion of food left over from feeding after the infected food had disappeared, was removed from the hutch.

Result:

The rabbit was found dead at 7 a.m., September 3rd. *P.M.*, *Negative.*

(c) August 30th, 10.15 a.m.

A half-grown rabbit was placed in a hutch, in which a rabbit fed, August 28th, upon cabbage-leaves with 2½ ccm. of virulent broth-culture, was found dead (from "chicken-cholera") at 9 p.m., August 29th. In the hutch there was a considerable quantity of normal-looking droppings; part of the bottom was damp with urine. The rabbit which occupied the hutch before, had eaten up all the infected food given to it, August 28th; but some of other (uninfected) food given later on, was still in the hutch, and not touched when the fresh rabbit was placed in it.

Result:

The rabbit was found dead at 7.15 a.m., September 6th. *P.M.*, *Negative.*

(d) September 4th, 2.50 p.m.

A rabbit, not quite full-grown, was placed in a hutch in which a rabbit, fed the day before on cabbage-leaves with 1 ccm. of virulent broth-culture, was found dead (from "chicken-cholera") at 2.40 p.m., September 4th. With regard to amount and appearance of excrement and food in the hutch, about the same state was noticed as in the foregoing experiment.

Result:

The rabbit was found dead at 9.15 a.m., September 9th. *P.M.*, *Negative.*

(e) September 4th, 2.50 p.m.

A young rabbit was placed in a hutch in which a rabbit, fed the day before upon cabbage-leaves with 2½ ccm. of virulent broth-culture, was found dead (from "chicken-cholera") at 2.40 p.m., September 4th. Amount and appearance of evacuations and food in the hutch, as before.

Result:

The rabbit was found dead at 9.15 a.m., September 9th. *P.M.*, *Negative.*

Some direct experiments were made with fæcal matter, or contents of the cæcum (the first and most voluminous portion of the large intestines) from rabbits which succumbed to typical "chicken-cholera" consequent on feeding. They showed that such material (in rectum and in cæcum) contained enough active bacteria to cause rabbits to perish from undoubted "chicken-cholera" when they were *inoculated* with small portions of that material. On the other hand, feeding on considerably larger quantities along with green stuff proved altogether inefficacious. The experiments may be described as follows:—

1888.

(1) Contents of lowest portion of rectum of a rabbit which, having died from "chicken-cholera" (feeding on virulent material) in one of the burrows (mentioned), was not removed therefrom after about four days. As an exception, the rectum contained very soft coherent green fæces. These were derived for examination by means of a sterilised, blunt glass-tube carefully introduced into the anus.

(a) *Inoculation.*

September 2nd, 11.35 a.m.

A rabbit was inoculated, subcutaneously at the belly, with a medium-sized platinum-loop full of such material. It was observed to die at 10.50 a.m., September 3rd, *i.e.*, 23¾ hours afterwards. The symptoms of the animal when dying, and the subsequent autopsy, together with microscopical examination of the blood, clearly proved the diagnosis—"chicken-cholera."

(b) *Feeding.*

September 3rd, 8.15 p.m.

A half-grown rabbit which had not been fed since 9 a.m. of the same day, was given a few cabbage-leaves smeared over with nearly

1 gramme of the fæcal matter, kept moist since the preceding day under a bell-jar. The rabbit, when seen at 10 p.m., had eaten all the food. It, however, did not show anything abnormal afterwards. It remained intact from this treatment.

(2) Contents of cæcum from a young rabbit, dead from "chicken-cholera" (feeding on virulent material). The cæcum was laid open at one spot by means of a hot scalpel, and the matter cautiously collected in a sterile test-tube.

October 15th, about 11 a.m.

Two half-grown rabbits which had not received any food in the morning, were given, together in one hutch, cabbage-leaves to which were attached 5 ccm. of a mixture consisting half of the above material and half of a 0·6 per cent. sterile salt-solution. They had eaten all but a few small pieces of leaves at 12.30 p.m., and had quite finished eating when seen at 2 p.m.

Results:

One was observed to be dying at 8.10 p.m., October 16th. As it was paralysed and evidently in pain, it was then killed.

The other was found dead at 9.15 p.m., October 18th.

In both instances the *post-mortem* examination yielded a negative result with regard to "chicken-cholera."

(3) Contents of cæcum from a robust full-grown rabbit shortly after its death from "chicken-cholera" (feeding on virulent microbes). Material derived as before.

1889.

(a) *Inoculation.*

May 6th.—One full-grown healthy rabbit inoculated (as before) at 4.45 p.m. with a medium-sized platinum-loop full of such material from cæcum. It died at 7.25 p.m., May 7th, under characteristic symptoms. The subsequent *post-mortem* examination secured the diagnosis—"chicken-cholera."

(b) *Feeding.*

May 6th.—One full-grown healthy rabbit was given, shortly after the above time, some bran with which were mixed 2 grammes of the matter of the cæcum-contents, diluted with some 0·6 per cent. sterile salt-solution.

The rabbit, being still lively on May 20th, was taken from its hutch and turned loose in an enclosure with others. According to

the caretaker on Rodd Island, it was found dead there, June 6th. It had been burnt when I visited the Island. His description of the condition of the carcass did not lend any support to its having succumbed to "chicken-cholera."

Besides, the result of another experiment showed that urine, taken from a rabbit newly dead in consequence of inoculation with virulent culture, had no effect on fresh rabbits which were inoculated with it.

1888.

September 11th, 1.30 p.m.

Two rabbits received subcutaneously about $\frac{1}{8}$ ccm. (2 minims) of such urine. The bladder of the rabbit from which the latter was obtained was much distended. The deep amber-yellow urine, which contained much firm matter (urates), was derived by means of sucking a small portion into a sterile glass-tube, through a little hole made by a hot glass rod into the lifted and stretched vertex of the bladder.

Results :

One rabbit was found dead at 7.30 a.m., September 13th. *P.M.*, *Negative.*

The *other* remained alive.

Transmission of the Virus of Chicken-cholera through Rabbits in Successive Generations.

It being from a theoretical as well as from a practical point of view—in case the microbes of chicken-cholera were to be employed as a means for the destruction of rabbits in Australasia—a matter of some importance to know whether these microbes, by passing through the bodies of rabbits in a number of continuous generations, become altered in their degree of virulence or not, it was decided that such an experiment, with a view to obtaining the required information, should be made, extending to the number of twenty successive transmissions from rabbit to rabbit.

Let us suppose the virus under consideration is endowed with the faculty of becoming more virulent, or, in other words, of

attaining a greater poisoning strength in its action on rabbits, by means of such successive transmissions, the consequence will naturally be that, under the same conditions, the period of incubation and actual disease, or the whole period from infection to death, becomes shorter, until a certain stationary point is reached; this period must, on the other hand, provided the conditions be the same, become longer, or infection with subsequent death may not follow at all, if there should be any decrease or attenuation of the virulence of the microbe. Should the latter preserve its degree of virulence uniformly from the first rabbit to the last, it stands to reason that the above period will remain about the same throughout, provided again the conditions be the same.

The experiment was carried out in the following manner:—A healthy pigeon was inoculated, October 3rd, with a small quantity of the surface-growth of a virulent stick-culture of the microbe of chicken-cholera (fourth generation, 32 days old). Not long after the death of this pigeon, which died of typical chicken-cholera within about twenty hours, two rabbits were inoculated, each with five platinum-loops full, equal to $\frac{1}{40}$ ccm., of heart-blood from this pigeon. The blood of the *first* rabbit that died, or that was found dead, furnished the material for inoculation, in like manner, into two further rabbits; with the blood again of the *first* of these dead, two other rabbits were inoculated, and so on till the number of forty rabbits, or twenty generations, were arrived at, when the experiment was concluded.

Before directing attention to the table of results of the experiments given below (Table III., at the end), I wish to state the following:—The rabbits used were, if not specially noted to the contrary, full-grown animals of normal appearance. If at the *post-mortem* examinations anything abnormal was found, it will be remarked in that table. The rabbits for this experiment were taken irrespectively of the sex. From practical reasons it was not possible to employ either males or females from the beginning to the end.

Immediately after the inoculations, the rabbits were placed in spacious clean hutches, separately, and food was given to them as usual. They were also, all of them, sheltered from rain and sun in like manner.

The blood used for the inoculations was in each case derived from the right *atrium* of the heart, near the *venæ cavæ*. The quantity of blood derived was pretty uniformly the same each time, viz., $\frac{1}{40}$ ccm. (*vide* above).

The time of inoculation of each new series lay within about *two* hours from the moment the first of the preceding series died. In cases where such rabbits were found dead, instead of being observed to die, the body-temperature then taken yielded a cue as to the approximate time when death occurred.

The seat of inoculation was always a corresponding area on the left side of the belly. After having shorn this area, a small fold of the skin, where there was no blood-vessel running, was cut across by means of a small pair of scissors. The wound thus produced was made the entrance into a small subcutaneous pouch, where the inoculation-material was easily and safely deposited by means of the platinum-loop.

The quantity of bacteria thus inoculated into the different rabbits was, comparatively speaking, a limited one. The direct microscopical examination of uniformly obtained and stained samples of heart-blood of all the rabbits shortly after their death, succeeded in showing only moderate numbers of individual bacteria.

In four cases I have tried to determine approximately the number administered, namely, in Inoculation Series x., xv., xix., and xx., Nos. 19, 29, 37, and 39, Table III. About 10 ccm. of 6 per cent. rabbit-broth-peptone-gelatine in a test-tube were liquefied, so as to have a temperature of between 30° and 40° C., mixed with *one* platinum-loop full (one-fifth of the quantity for inoculation) of the heart-blood, and made to solidify, by means of iced water, in a homogeneous layer along the inner walls of the test-tubes (Esmarch's method). After having been in a thermostat at a suitable temperature for three or four days, the coating of gelatine in the tubes presented innumer-

able, as it seemed, whitish points of growth, or colonies, the number of which, however, could without difficulty be calculated by counting the number of colonies at, usually, ten different spots each of the area of one-sixteenth of a square cm. area, cut out of a piece of black paper. The total number of colonies which were calculated as being contained in the four tubes, amounted to 67623, 71887, 65367, 48593, *i.e.*, in the mean, 63368. This figure multiplied by five yields 316840, and, if we are permitted to make use of this average number, we may well say that the quantity of microbes transmitted into the rabbits along with blood, was not very far off this number. We are, however, well justified in taking it somewhat higher, from the fact that a portion of the bacteria in the blood are occurring in twos, which will not be easily separated by mixing with gelatine, in which they will give rise to but one colony.

This relative scarcity of the micro-organisms of chicken-cholera in the heart-blood of rabbits, *newly dead*, stood in a sharp contrast to the relative abundance of these microbes in samples of the same blood, taken from rabbits *which had been left* where they died untouched, (say) for *twelve*, *twenty-four*, *thirty-six*, or *more hours*. In each case, where such a comparison was made — for that purpose one portion of the rabbits, as used for the inoculations, were examined soon after their death, and the other corresponding portion at some time after their death; but also on other occasions, when I had an opportunity of thus comparing,—I could not fail to be struck with the disparity of the heart-blood alluded to.

On the other hand, it was repeatedly noticed that the capillary-blood derived from cut surfaces of the liver, soon after the death of the rabbits, contained incomparably more numerous bacteria than the heart-blood derived from the same subject, and at the same time.

I must add that all the samples for microscopical examination were derived in like quantities, and spread and stained on cover-glasses in like fashion. From this it is evident that in rabbits dead of "chicken-cholera," at the time of death or shortly after it, the blood of the heart and main vessels carries only a relatively small number of the bacteria, and that their relatively plentiful

occurrence there, some such time after death as noted above, can only be declared by their having multiplied there after the death of their hosts.

The table, the arrangement of which will, I think, be easily understood, contains the results of this experiment. I may at once remark that, for the sake of convenience, those rabbits which as having died first were used for the successive inoculations of the different series (column one), are designated uniformly by the first (odd) number (column two) of each series. (See Table III., at the end.)

In looking over the figures in this table, we cannot help arriving at the conclusion that by transmitting the virus of chicken-cholera from rabbit to rabbit, to the extent of twenty generations, neither an increase, nor a decrease in its virulence is attained—that, rather, its virulence does not exhibit any striking differences throughout the whole series. It is true that in four cases out of the forty, the figures regarding time of death are a little lower than usual (Nos. 1, 21, 29, 33), that in three other cases they are somewhat higher (Nos. 4, 14, 28), and that in one case (No. 34) the figure is very high. But these exceptions may be declared from certain individual properties of the rabbits employed. It was, as a matter of fact, not possible to take exclusively only such rabbits as were like one another in every respect (age, size, weight, sex, and health).

That the rabbit No. 34, Series XVII.—a light-grey female, with a white streak running longitudinally from the back of the head over the middle of the head down to the underside of the neck (mammary glands fully developed, containing milk)—did not succumb until two days after inoculation, which had been performed in the usual manner, is very remarkable. Seeing it outlive the first day, I thought of having hit upon another example of immunity in rabbits. The *post-mortem* examination later on left no doubt as to its having died of "chicken-cholera." I may, however, mention that the seat of inoculation differed from that in all rabbits inoculated, in so far as there was a yellowish-white membraneous formation adhering to

the under surface of the cutis, of about the size of a sixpence. This appearance was not unlike that which is noticed at the seat of inoculation with the virus of chicken-cholera in the case of fowls and pigeons; but while the substance of the yellowish subcutaneous masses forming after inoculation in fowls and pigeons are found to be crowded with chicken-cholera bacteria, in the corresponding case of the rabbit these bacteria were exceedingly scarce.

Tests of Virulence with regard to Fowls and Pigeons.

The next table (IV.), at the end, contains the results of inoculations into fowls and pigeons, with heart-blood from the first-mentioned rabbits as used in the Inoculation Series v., x., xv., xx., of Table III. In reference to the first case which I denoted as inoculated from Inoculation Series i., I must state that, as the date (16th October) implies, that particular rabbit was not exactly the first dead of the first generation as followed directly by the others; this experiment was added later on, when, on 14th October, a pigeon was inoculated with a small quantity of the surface-growth of a gelatine stick-culture of the microbe (fifth generation, 17 days old), and after the death of the pigeon, which died between 14 h. 15 m. and 17 h. 5 m., a rabbit was inoculated (15th October) in pretty much the same way as the two of the first series in Table III. From this rabbit, which died between 12 h. 30 m. and 15 h. 45 m., the fowl and pigeon of Series i. of the following table were inoculated. Therefore, I call Series No. i. simply inoculated from Inoculation Series i. The conditions under which the five series were inoculated, were, on the whole, corresponding to those stated for the rabbits (Table III.); the seat of inoculation was an area under the skin which covers the pectoral muscle. (See Table IV.)

As evidenced by the data obtained and put together in this table, the virulence of the microbes of chicken-cholera neither increases nor decreases, perceptibly, in fowls and pigeons inoculated with virus descending from rabbits of the first, fifth, tenth, fifteenth, or twentieth Inoculation Series. The hours which it

required to kill either fowls or pigeons, did not show any considerable difference in either the one or the other case, so that we may say here, as we did before, that the degree of virulence was at the end of the experiment practically the same as at the beginning.

Notes on the Body Temperatures of Rabbits inoculated with the Microbes of Chicken-cholera.

In connection with the experiment conducted with a view to determining the degree of virulence of the bacteria of chicken-cholera, when made to pass though the bodies of rabbits in twenty generations of two rabbits for each, I have been able to make a series of observations regarding the body-temperatures of such rabbits.*

In a number of cases the temperature was taken, at intervals, from the time of inoculation (immediately before it) until death (immediately after it); in several instances only up to some time before death.

These observations are put together in Table V., at the end.

From the data given in this table we may reasonably conclude that—

1. As *a priori* intelligible, the septicæmia which is the result of transmitting virulent chicken-cholera bacteria into rabbits, is associated with a gradual increase in the body-temperature, which in its maximum was found to differ from the initial temperature by 2·5° C. in one case (No. 8), by 1·95° C. in another (No. 2), by 1·9° C. in a third (No. 7), by 1·8° C. in two others (Nos. 10 and 16), while in the remaining cases the difference was less.
2. This maximum, as a rule, is noticed some little time before death.

The difference between the initial body-temperatures (taken immediately before inoculation) and the final body-temperatures (taken immediately after death) may also be seen, in the

* The observations were made by means of an ordinary clinical thermometer which, after having been oiled with *ol. amygd. dulc.*, was introduced into the anus to the length of between six and seven centimètres.

majority of cases, as noted in the aforementioned table. Apart from these, I can offer several examples where only initial and final temperatures were taken. All the examples that may thus be utilised, number nineteen. The following are the figures as compared with one another in the different instances :—

40·0 —39·6 :	39·2 —39·67 :	39·6—40·6 :	39·3—41·0 :
38·9 —39·6 :	39·1 —40·2 :	39·2—39·15 :	39·4—40·0 :
39·05—39·45 :	38·85—40·0 :	39·1—40·9 :	39·8—39·6 :
39·8 —41·25 :	39·0 —39·9 :	38·8—39·65 :	39·9—41·7 :
39·8 —40·2 :	39·6 —41·2 :	40·0—38·4 :	

In fifteen cases, then, out of nineteen, the body-temperature was found to be higher at the end than at the beginning. Taking the mean out of each of these two series of fifteen observations, we arrive at the figures 39·3—40·355 ; the difference is thus 1·055° C. In four cases out of the above nineteen the final temperature was lower than the beginning. Taking, again, the mean out of each of the two series of four observations, we obtain the figures 39·75—39·19, that is a difference of 0·56° C. in favour of the final temperature.

From this, therefore, we may deduce that, as a rule, the final body-temperature is higher than the initial by about 1° C. on the average.

I may add the initial temperatures of eleven more cases, without any corresponding final temperatures. They were 39·9, 39·85, 40·2, 40·0, 39·45, 39·3, 40·1, 39·4, 38·7, 39·0, 39·6. The final temperature in one case, without any initial temperature taken, was 41·25.*

Although the temperatures which the air showed during the course of the experiments recorded above (Table III), may be regarded as having only a secondary meaning in the judgment of the results obtained, it is just as well to give a number of figures as they were noted.† They show here and there marked differences (see next page) :—

*Note on the Respiration.—The breathing, shortly before death, is very much accelerated. In one instance, two minutes before the death of the rabbit, I have found it to be forty-six to ¼ minute ; in another instance, ten minutes before death, forty-four to ¼ minute.

† These figures are also put down in connection with certain statements as to the relative quantity of bacteria in the blood of rabbits, some time after their death of "chicken-cholera," *vide* above.

October 4th.	1.35 p.m. 25°	3.35 p.m. 25½°	5.35 p.m. 22½°	7.35 p.m. 20°	9.40 p.m. 18¾°			
,, 5th.	2.10 p.m. 21°	4.10 p.m. 19¼°	6.45 p.m. 17¼°	8.55 p.m. 16°	11 p.m. 14½°			
,, 6th.	2.5 a.m. 11½°	5.40 a.m. 9½°	10 a.m. 19°	12.10 p.m. 22¼°	2.15 p.m. 19¾°	4.15 p.m. 18¾°	7 p.m. 16½°	8.40 p.m. 15½°
,, 7th.	9 a.m. 18¾°	10.40 a.m. 21¾°	12.40 p.m. 26¼°	2.50 p.m. 24¼°	4.50 p.m. 20¾°	6.55 p.m. 19½°	9 p.m. 19°	11.10 p.m. 19°
,, 8th.	1.30 a.m. 16¼°	4 p.m. 13¾°	5.5 p.m. 12°	7.30 p.m. 12°	8.55 p.m. 14°			
,, 9th.	8 a.m. 14¾°	10.55 a.m. 16°	1 p.m. 15½°	3.10 p.m. 16¾°	5.10 p.m. 15¼°	7.20 p.m. 14¼°	9.20 p.m. 14°	11.31 p.m. 14½°
,, 10th.	10.30 a.m. 17¾°	12.50 p.m. 18¼°	3.20 p.m. 17½°	5.55 p.m. 15¾°	8 p.m. 15½°	10.15 p.m 12¾°		
,, 11th.	12.55 a.m. 12½°	10 a.m. 19¼°	12.15 p.m. 21¾°	3.30 p.m. 21¾°	8.20 p.m. 16¼°	10.10 p.m. 15½°	11.43 p.m. 14°	
,, 12th.	12.47 a.m. 12½°	7.45 p.m. 17½°						
,, 13th.	8.10 a.m. 17¾°	10.40 a.m. 21¾°	1.5 p.m. 23½°	6.10 p.m. 18¾°	8.10 p.m. 18°			
,, 14th.	1.15 a.m. 15¼°	12.50 p.m. 25½°	3.25 p.m. 23¼°	6 p.m. 18°	8.10 p.m. 17½°	10.15 p.m. 17½°		
,, 15th.	5.40 p.m. 18°	8.10 p.m. 17°	10.30 p.m. 15¾°					
,, 16th.	3.5 a.m. 12¾°	7.20 a.m. 15°						

Experiments on Indigenous Birds.

In the appended Table VI.—*(a, b, c, d)* are put together, seriatim, the results of experiments with the microbes of chicken-cholera on a number of indigenous birds. These consisted of :—

(1) Two wekas, or Maori- or wood-hens *(Ocydromus australis*, Sparrm). *Habitat :* South Island of N.Z.

(2) Two magpies *(Gymnorhina tibicen*, Lath.). *Hab. :* Q., N.S.W., V., S.A.

(3) Two laughing-jackasses *(Dacelo gigas*, Bodd.). *Hab. :* Q., N.S.W., V.

(4) Two butcher-birds *(Cracticus torquatus*, Lath.). *Hab. :* Q., N.S.W., V., S.A.

(5) One blue-jay *(Graucalus melanops*, Lath.). *Hab. :* Austral. (and New Guinea).

(6) Two gallahs, or rose-breasted cockatoos (*Cacatua roseicapilla*, Vieill.). *Hab. :* Austral.

(7) Two wonga-pigeons *(Leucosarcia picata*, Lath.). *Hab. :* Q., N.S.W., V.

(8) One bronze-wing pigeon *(Phaps chalcoptera*, Lath.). *Hab. :* Austral.

(9) Two common swamp-quail *(Synoicus australis*, Lath.). *Hab. :* Austral.*

[See Table VI. *(a), (b), (c), (d)*, at the end.]

(10) Six crows *(Corone australis*, Gould). *Hab. :* Austral.

(See p. 560.)

* All the specimens of birds mentioned under 1—9, were obtained, in an apparently good condition, from a dealer at the Sydney markets, on the 8th October. On the Island they were kept in spacious, airy boxes, so as to be protected from any injurious effects of the weather. When they were to be experimented upon (in case of feeding only), they were slightly starved beforehand, and their boxes emptied of all except water. During the course of the experiments they were regularly fed, as usual.

From the results so far obtained we see that the virus of chicken-cholera, derived, as it was, in the shape of blood from rabbits which died in consequence of infection by that virus, proved, when caused to gain entrance into the digestive organs in the noted quantities, fatal to the magpies, butcher-birds, and blue-jay (which are principally animal feeders), to the wonga- and bronze-wing pigeons, to the gallahs and quail (which are all of them vegetable feeders). One of the wonga-pigeons, however, and one of the quail, did not succumb until after having been fed a second time on somewhat larger portions of the virus than before. One of the gallahs, although surviving two experiments by feeding, perished quickly in consequence of inoculation, thus manifesting its ready susceptibility to inoculated chicken-cholera.

Of two laughing-jackasses (true animal-feeders), one died after the first experiment (feeding), but not of chicken-cholera, as shown by the result of the *post-mortem* examination. The other survived feeding on virulent material for two successive times; but when inoculated later on, it succumbed, we are entitled to say, to this disease, in so far as evidenced by the occurrence of numerous bacteria of chicken-cholera in the blood, by their successful cultivation, and inoculation into a healthy rabbit, which died as usual. The appearance of the organs was less characteristic than is usually the case with birds dead of the disease.

Two wekas (animal-feeders), of which one was once fed and twice inoculated, the other twice fed and once inoculated, remained alive. Whether their insusceptibility arose from the fact of their having been possibly treated preventively at first, or whether—what seems to me to be not at all impossible—birds of this description are naturally immune against chicken-cholera in any shape of application, can only be decided by further experiments.

Nearly five and a half months later, the two wekas (rooster and hen) were subjected to a last inoculation, this time of a considerably larger quantity of virulent blood from a "chicken-cholera" rabbit [see Table VI. (*d*)]. The result was that the weka-hen

remained alive for good, whereas the rooster was found dead 42 hours after inoculation (having died in less time than that). The inoculation, in this instance, had not run off smoothly; instead of applying, as intended, the same quantity as that injected into the hen (in each case under the skin of the right side of the breast), only about half of that penetrated under the skin: the animal may have become too much injured at the place of inoculation, in consequence of the manipulation. At the *post-mortem* examination, the seat of inoculation and neighbouring portions were in a state of hæmorrhagic infiltration. The organs presented everywhere indications of general sepsis. The blood, of black colour, showed in cover-glass preparations a moderate number of bacilli which, although being larger than the chicken-cholera bacteria usually are, resembled them. There were also bacilli of a different form. In order to arrive at a certainty whether the former were true chicken cholera bacteria, and active, I inoculated a medium-sized fresh rabbit with heart-blood of the weka-rooster. The rabbit was found dead 20 hours afterwards, it having died between $10\frac{3}{4}$ hours and that time. The finding of the examination was: death from typical "chicken-cholera." Notwithstanding this occurrence of virulent bacteria in the heart-blood of the weka-rooster—they were also observed in the spleen—it is very doubtful whether this case is to be placed under the heading of a true infection by those microbes. To judge from the *post-mortem* appearances, I think, the presence of these microbes in the vascular system might be explained without adopting the view of an infection, properly speaking.

That with regard to all the representatives of indigenous birds which, experimented upon, died, the cause of death must be regarded as due to chicken-cholera, as briefly noted in the quoted table by "*P.M., Positive*" (with the exception of one laughing-jackass, where the *post-mortem* was negative, and very likely of the weka) was, I think, conclusively demonstrated by the presence, usually in immense numbers, of the typical bacteria in the blood; by cultivation of such material in suitable media, when they gave rise to typical cultures; and by the positive results of occasional

inoculations of blood into normal rabbits. Besides, the appearance of the organs was nearly always such as bearing a close resemblance to that in the case of fowls and pigeons which succumb to the disease.

Certain results obtained in the foregoing experiments, would seem to lead to the belief that indigenous birds, as exemplified by a few instances, may not always necessarily become affected or killed by taking up, along with food, certain small or minute quantities of the microbes derived, we had better add, directly from the bodies of rabbits newly dead of "chicken-cholera." On the other hand, inoculation with the virus taken from the same source, may be looked upon as a far more dangerous, although naturally more rarely occurring, mode of infection for such birds. Further below I shall mention a corresponding case in common pigeons.

(10.) Indigenous Crows.

At my request, Mr. Taylor, of the Rabbit Branch, Lands Department, Sydney, caused a number of indigenous crows to be caught near Hay, New South Wales, and to be forwarded to me. On the 8th and 10th November, 1888, I received them, eight in all, of which, however, two died soon after arrival. The remaining six appeared in good health, although at first they were a little sluggish. They belonged to the species *Corone australis*, Gould; found all over Australia, including Tasmania. I am told that there is very little difference between the two species of crows described from Australia; one is the above-mentioned, and the other is *Corvus coronoïdes*, Vig. and Horsf., which is said not to occur in Tasmania.

I enumerate the experiments upon the six crows in chronological order:—

1888.

(i) November 13th, 11 a.m.

Two of the crows, kept in one box with plenty of space in it, were inoculated (under the skin over the pectoral muscle on one side) with fresh virulent liver-blood taken from a rabbit which died of "chicken-cholera" on inoculation.

One received 1-16th ccm. = 1 minim
The *other* received 1-32nd ccm. = ½ minim } of such blood.

Results:

November 15th.—The *one* which had been inoculated with 1 minim of blood, was found dead at 7.30 a.m. *P.M.*, *Positive.* (Appearance of the organs resembling to some extent that of the organs of poultry dead of chicken-cholera. Immense numbers of typical bacteria in the blood.)

December 1st.—The *other* which had received only ½ minim of blood, was still alive on this date, when it was used otherwise, as will be seen below.

(ii) November 13th, 12.35 p.m.

Four crows which were accommodated in a commodious specially-fitted stall in the shed, and which had not been treated so far, were fed on the livers of two rabbits which had succumbed to "chicken-cholera" on feeding.

Annotations:

(1) The crows, although not being fed on the morning of that day, were very slow in eating the pieces of liver placed in their stall on a soup-plate.

(2) The feeding had, from want of rabbits at the time, to be discontinued until later (*vide* below).

Result:

December 1st.—The *four* crows were still alive.

(iii) December 1st to 7th.

In the stall which contained the above four crows, and into which was turned the one which had been inoculated previously with ½ minim of virulent blood, were placed, for *seven* consecutive days, in the mornings, the carcasses, each time, of two rabbits which died of "chicken-cholera" on inoculation (in connection with the desiccation experiments (p. 572).

Annotations:

(1) The dead rabbits (*fourteen* in all), before being given to the crows, had been deprived of their entrails (with the exception of liver, kidneys, heart, and lungs), and as there were more on hand than were required at the time, they were kept in a cool place, so that the carcasses were still fresh when placed in the crows' stall, with the exception of one (out of the last feeding) in which putrefaction had already set in.

(2) During the above-mentioned period the crows did not receive any other food. Water, of course, was always provided.

Results:

December 4th.—*One* found dead at 9.30 a.m. (alive at 8.15 a.m.). *P.M.*, *Positive.* (Carcass stiff; blood coagulated, and of a tarry appearance; hyperæmia of intestines; contents of small intestine consisting of slimy, yellowish masses, stained here and there with extravasated blood; spleen apparently enlarged, cherry-brown. Immense numbers of bacteria of chicken-cholera in the blood. A healthy rabbit inoculated with a small quantity of such blood, succumbed promptly. Cultures derived from blood of this rabbit were further tested, so that with regard to this crow there cannot be any doubt as to the cause of its death).

December 9th.—*One* found dead at 8.30 a.m. *P.M.*, *Positive.*

The *three* remaining crows, among them the previously inoculated one, were still alive on

(iv) December 14th, when, at noon, they were inoculated with fresh virulent blood derived from the liver of a rabbit that died of "chicken-cholera" on inoculation. Of these three crows, two, of which one had been inoculated before, received 1 minim each of the blood; the third $\frac{1}{2}$ minim.

Results:

All three crows remained alive and well, thus showing that they were altogether refractory to this treatment.

1889.

(v) April 8th, about noon.

After a lapse of nearly four months, the three crows were inoculated again, at the above time, with liver-blood from a rabbit recently dead from "chicken-cholera"; each crow received the rather large dose of $\frac{1}{4}$ ccm. (4 minims) of such blood injected under the skin of the left side of the thorax.

In the evening of the same day, and at noon of the following day, they were seen to have eaten only portion of the meat given to them.

Results:

One crow which some time ago had lost one of its feet through injury, died between 3 p.m. and 3.15 p.m., April 9th; 27 hours after inoculation. The carcass was found resting on a perch, and its head leaning against the wall.

A *second* crow which looked dull, and ruffled in plumage, in the evening of April 9th, was found dead at 6.25 a.m., April 10th; it was lying on the floor of the stall.

The *third* crow which also was ill since the previous evening, was found dead at 7.30 p.m., April 10th; lying on the floor; it must have died between 5.50 p.m. and that time.

(A vigorous full-grown rabbit, also inoculated with $\frac{1}{4}$ ccm. of that liver-blood, as control, was found dead at 7 a.m., April 9th. It must have died soon after 10 p.m., the previous night.)

The examination of the carcasses of the three crows, of which the last two were in a very good condition, resulted in showing that they all had succumbed to chicken-cholera. The carcasses were very stiff. At and round the seat of inoculation there was, in the case of the last two crows, a tough, yellowish-white formation, resembling in appearance what is known in fowls or pigeons similarly treated. Spleen conspicuously enlarged, cherry-brown, and soft. Intestines hyperæmic; hæmorrhagic exudations in the duodenum of the crow which died first. Blood mostly coagulated, blackish; in it innumerable numbers of the typical bacteria of chicken-cholera.

To judge from the outcome of these experiments we may say, generally, that the microbes of chicken-cholera are only under certain conditions fatal to crows. Small doses of the virus, it appears, are not efficacious enough to become fatal; on the other hand, repeated feedings on larger quantities of virulent material are more dangerous, while inoculations with larger quantities of such caused death (from chicken-cholera) each time. The previous treatment of the crows mentioned under iii and iv, may have had something to do with the surviving of the greater portion (iii), or of all of them (iv). These treatments combined, were, however, unable to protect—if there was any protection at all—the three crows, when they were subjected to a severer test, about four months later.

How far there is danger for all the useful indigenous birds to take up the disease (chicken-cholera), should the latter be introduced into the country for the sake of rabbit-destruction, cannot be precisely defined from the results of the above experiments. That such a danger, however slight it may be, does exist, if the disease was intentionally spread and reared in the open, cannot be denied by the unprejudiced mind; and that, even admitting that in the

first instance only a minute fraction of wild birds may be carried off by the disease—an occurrence which in itself would be of little importance—these few birds, travelling as they may, perhaps, after having become infected, may transmit the germs hither and thither, ready to be taken up again by susceptible birds of the same or some other description.

Experiments on Common Fowls and Pigeons.

(a).

Feeding and Inoculation.

On page 553 and Table IV. (at the end), I have already recorded certain inoculation-experiments with reference to common fowls and pigeons. This was in association with the experiments on the behaviour of chicken-cholera bacteria when removed from rabbit to rabbit through twenty generations.

Table VII. (at the end), (*a*, *b*, *c*, *d*, *e*), contains an account of the arrangement and the results of other experiments.

From it will be seen that one fowl (hen) proved insusceptible to taking chicken-cholera by feeding on a small portion of virulent material from a dead rabbit, while after a second feeding on a considerably larger portion it died, unfortunately, soon afterwards, from some cause different from chicken-cholera. (The result of the *post-mortem* examination is denoted as *negative* in the table). Another fowl (heavy rooster) was fed three consecutive times on successively larger portions of virulent material (taken from rabbits) without the least harm to its health. Later on it was inoculated with a small quantity of active microbes, but it remained alive. [Necrotised tissue was thrown out where the seat of inoculation was, corresponding to what takes place in fowls which are treated preventively with attenuated virus of chicken-cholera (Pasteur)].

The immunity of the rooster, in this instance, was possibly due to the animal having undergone three previous and successive feeding experiments, which might have had a protective influence.

Nearly five months and a-half later, the rooster, which was then very robust, received subcutaneously (breast) a much larger quantity of virulent rabbit-blood [see Table VII. (at the end) (*e*)]. This time the rooster did not resist; it died, under the typical chicken-cholera symptoms, 27 hours after inoculation, after a short illness. The *post-mortem* examination revealed an example of severe chicken-cholera. The duodenum was filled with almost one mass of blood.

Two pigeons which were repeatedly fed (the one twice, the other three times) on food contaminated with active microbes, succumbed promptly to the effects of inoculation later on, thus showing that they had not been rendered immune by the previous treatments. However, in the judgment of these results, it should be borne in mind that, as the pigeons were too slow in eating (see Table VII.), the preceding treatments (feeding) cannot be regarded as exact. (The results of the *post-mortem* examinations are simply denoted as *positive* in the table.)

(b).

Experiment with a view to ascertaining the effect of exposing poultry to rabbits which are dying from "chicken-cholera" (after feeding), and the carcasses of which are allowed to remain with the former for some time.

For this purpose the Aviary on the Island, shortly described in the Introduction, was utilised. At the beginning of the experiment, November 9th, 1888, it contained nine fowls, (of which three had been there for some time, as left over from a former consignment of twelve, and six had been received from the Sydney Markets the day before, November 8th), and twelve pigeons, also obtained from the Markets on the latter date. Neither the fowls nor the pigeons had so far been experimented upon in any way.

. The experiment, as already mentioned, was begun November 9th, and lasted five weeks, up to December 14th.

Within this period rabbits were introduced, at intervals, in all three times.

1888.

(i) November 9th, 10 a.m.

Two rabbits, one quite full-grown, the other nearly full-grown, were given cabbage-leaves sprinkled with 3 ccm. of an active broth-culture for each rabbit. When seen at 12.15 p.m., they had finished their portions of infected food.

At 12.30 p.m. they were let go in the aviary.

Results:

One observed to die at 10 a.m., November 10th; the other about two hours later, at 12.7 p.m., both under "chicken-cholera" symptoms. Their carcasses also showed the typical stiffness. A control-rabbit which was found dead at 7.30 a.m., November 10th, was, on examination, proved to have succumbed to "chicken-cholera."

(ii) November 22nd, 11 a.m.

Three full-grown rabbits, having besides others arrived on the Island on the previous day, were given cabbage-leaves with 2 ccm. of a fresh broth-culture for each rabbit (at the same time six other rabbits were similarly fed, see p. 538). The three rabbits which were very slow in eating, although they had been left without food for some time, were placed in the aviary at 7 p.m.

Results:

One rabbit found dead at 7 a.m., November 23rd. As check for its having died from "chicken-cholera" may be taken a rabbit which, being among the six mentioned (turned into the main enclosure), was also found dead at 7 a.m., November 23rd; a sample of liver derived from this rabbit, contained the typical chicken-cholera bacteria. The *two* other rabbits being still alive, November 27th, were taken out of the aviary that day.

(iii) November 28th, 11 a.m.

Three full-grown rabbits were given green barley-leaves sprinkled with 2 ccm. of a fresh broth-culture for each rabbit. Two of them were seen to have eaten their portions of infected food at noon, the third at 1 p.m. At 3.15 p.m. they were transferred to the aviary.

Results:

One found dead at 7.30 a.m., November 29th.

Another found dead at 6 p.m., December 1st; seen alive an hour before.

In these two cases a sample of liver was derived, as described pp. 538, 539. The microscopical examination yielded large numbers of typical bacteria.

The *third* being still alive, December 14th, was removed from the aviary.

Thus *five* rabbits died in the aviary from "chicken-cholera," *two* November 10th, *one* November 23rd, *two* November 29th

and December 1st, respectively. The carcasses remained there until December 14th (see above).

Now, with regard to the poultry, penned up in the same aviary, I have to state that within the five weeks there died : *six* pigeons and *two* fowls. However, early on Nov. 10th, *i.e.*, very soon after the beginning of the experiment, *two* pigeons which were ailing before, were found dead. Leaving these two out altogether, the mortality, and the result of *post-mortem* examination, is as follows :—

November 13th—*One* hen found dead at 7.30 a.m. *P.M.*, *Negative.*
November 17th—*One* pigeon found dead. *P.M.*, *Negative.*
November 29th—*One* rooster found dead at 7.30 a.m. *P.M.*, *Negative.*
December 7th—*One* pigeon found dead at 8 a.m. *P.M.*, *Negative.*
December 11th—*One* pigeon found dead at 8.30 a.m. *P.M.*, *Negative.*
December 14th—*One* pigeon found dead at 8 a.m. *P.M.*, *Positive.*
(Characteristic appearance of organs : immense numbers of chicken-cholera bacteria in blood. Rabbit inoculated with small quantity of this blood perished from "chicken-cholera" in less than 10 hours after inoculation).

Thus, it was only once, namely in the case of the last pigeon, that the disease was communicated.* This result appears to rabbits dying and dead from "chicken-cholera," is not great under indicate that the danger to poultry which are associated with those conditions.

It must be mentioned that during the term of the experiment both fowls and pigeons were observed to peck freely at the dead rabbits lying about. When the latter were removed ultimately, the three rabbits which had died first, presented only fragments scattered in different directions. The two which died last, were

* After December 14th, another death occurred in the aviary, namely that of a hen which was found dead at 7 a.m., December 15th. The result of the *P.M.* examination, and the successful inoculation of some heart-blood of this hen into a healthy rabbit, was undoubted proof of death being due to chicken-cholera. It cannot, however, be decided whether this hen died in consequence of infection from the dead rabbits which were in the aviary up to December 14th, or of infection from the droppings of the pigeon which, having been found dead on the last-mentioned date, was shown to have succumbed to chicken-cholera.

not dismembered, but they were pecked open, and their flesh and entrails mostly missing.

Experiments on Hares.

Below are recorded a few experiments with the chicken-cholera microbes on hares.* It will be seen that these rodents (which were employed in full-grown specimens) are as easily amenable to "chicken-cholera" as rabbits.

1888.

(i) August 11th, 3.30 p.m.

A hare was *inoculated* with five small platinum-loops full (about 1-40th ccm.) of virulent blood from a rabbit that had died after inoculation with a small quantity of a virulent broth-culture of the microbe.

Control :—A control-rabbit (full-grown) was found dead at 9 a.m., August 12th. *P.M., Positive.*

Result :

The hare was found dead at 9 a.m., August 12th. *P.M., Positive.*

(ii) August 11th, 4 p.m.

A hare was *fed* upon a few cabbage-leaves infected, by means of a platinum-loop, with about $\frac{1}{4}$ ccm. of blood from the same infected rabbit from which blood was taken for the inoculation of a hare this date [*vide* (i) above]. It was not until 10 p.m. (*i.e.*, six hours after the infected food had been placed in the box) that the hare was observed to have eaten all the infected food given to it.

Control :—A control-rabbit which had finished eating its portion of infected food shortly after the food was placed in its box, was found dead at 8.30 p.m., August 12th, having died between 5.30 p.m. and that time (*i.e.*, between $25\frac{1}{2}$ and $28\frac{1}{2}$ hours after being fed). *P.M., Positive.*

Result :

The hare was still alive at 11.30 a.m., August 16th (*i.e.*, about 8 days after the feeding referred to above).

(iii) August 16th, 11.30 a.m.

(*a*) The same hare was *fed* upon cabbage-leaves infected with $1\frac{1}{2}$ ccm. of a virulent broth-culture of the microbe.

---

* The hares used here were among five robust specimens received from the country, through Mr. H. C. Taylor, Rabbit Branch, Lands Department, Sydney.

(*b*) Another hare (which had survived from inoculation with some dried blood taken by Dr. Bancroft, of Brisbane, Q., from a hare that had died in captivity) was *fed* upon cabbage-leaves infected with 1½ ccm. of the same culture.

Control :

(*a*) Of *two* control-rabbits (large vigorous animals ; both *tame*†), *fed* together in the same box upon food infected with 3 ccm. of the same culture, *one* (a long-haired black specimen) was found dead at 8.45 p.m., August 18th (*i.e.*, about 57 hours after being fed). *P.M.*, *Positive.*

The *other* (a long-haired albino) was still alive on August 20th. (For further treatment of this particular rabbit *vide* pp. 523-525.)

(*b*) A control-rabbit (a tame long-haired albino) *inoculated* with a small quantity of the same culture, was found dead at 8 a.m., August 17th.

Results :

Both hares were found dead at 8 a.m., August 17th. *P.M.* (in each case), *Positive.*

Feeding of Guinea-pigs on Chicken-cholera Microbes.

About guinea-pigs it is said that, when inoculated with such microbes, they generally react by the formation, at the seat of inoculation, of closed abscesses which, as a rule, pass away again without being followed by a general infection and, as consequence, by the death of the animals. I have not made any inoculation-experiments, but, on the other hand, tried the effects of virulent microbes introduced into guinea-pigs through the alimentary canal.

On May 10th, 1889, at 2.30 p.m., 12 ccm. of a virulent broth-culture of the microbes—obtained from virulent heart-blood of a rabbit, and incubated for 24 hours at 37·75-37·9°C.—were uniformly sprinkled on, and made to adhere to, fresh cabbage-leaves which were placed in a large box

† Several tame rabbits (Angora) were sent to the Island in June, 1888, by Professor Watson, of Adelaide, S.A. Most of those which were not used for chicken-cholera experiments, exhibited "scab" later on, with which, I believe, they, or at least a portion, had been infected by Professor Watson.

containing five well-nourished guinea-pigs, namely : *three* young ones, about ¼-year old ; *one* (doe) not quite full-grown, about ½-year old , *one* (doe) quite full-grown. They all had been kept hungry for a while. At the same time, or rather a little before, a fresh rabbit was given 4 ccm. of the same broth-culture ; this rabbit which served in particular as control to two previously-treated rabbits, died about 24 hours after feeding (see pp. 528,529).

Results :

One guinea-pig, the ½-year old doe, died at 10.45 a.m., May 12th, the symptoms shortly before death being similar to those noticeable in "chicken-cholera" rabbits.

Another, the full-grown doe, died at 3 30 p.m., May 12th, in pretty much the same way as the preceding one.

The *three* ¼-year old guinea-pigs remained alive, somewhat to my surprise. They were watched for weeks afterwards, but were never seen to show any signs of illness. It should be mentioned that they were observed eating the infected food just as well as the two others which subsequently died, and that they must have partaken of it in proportion.

At the *post-mortem* examination of the two guinea-pigs, it was first noticed that *rigor mortis* was very well marked. On removing the skin at the belly, the veins were seen to be gorged with blood. There was a severe peritonitis and pleuritis, especially in the old guinea-pig. Heart distended with blood, which was of a blackish colour. Lungs very voluminous, reddish-white, here and there intersected with darker spots ; on section frothy, crepitating. Spleen enlarged, of apparently usual colour. Stomach filled with food. Intestinal canal very strongly hyperæmic ; in one case (full-grown female) the small intestine at different places containing blood-stained liquid masses ; in the other (younger female) the whole of the small intestine showing externally a dark cherry-red colour, and on being cut open, showing the contents consisting of liquid material very rich in blood. Something similar to such a degree of extravasation of blood into the intestines, I have occasionally met with in birds dead from chicken-cholera. The rectum contained solid, although soft, greenish fæces.

Cover-glass preparations of blood from heart and liver showed moderate numbers of "chicken-cholera" bacteria ; these were, on the other hand, exceedingly abundant in sap from cut-surfaces of the lungs.

A stick-culture, derived from heart-blood, in ordinary nutrient gelatine, was in its appearance exactly like others obtained from blood of rabbits or birds which died from "chicken-cholera."

Experiments on Ferrets.*

In the following is given the enumeration of experiments with chicken-cholera microbes on ferrets. Certain carnivorous animals, as dogs and cats, are already sufficiently known to be insusceptible to these microbes, and from the results obtained with regard to ferrets, it may reasonably be inferred that the latter are equally inaccessible to them.

1888.

(*a*) *Inoculation.*

(i) With *culture.*

September 10th, 11.30 a.m.

Two ferrets (one male, one female) were inoculated with $\frac{1}{8}$ ccm. of a virulent broth-culture of the microbe of chicken-cholera, obtained directly from blood of a rabbit that had died of "chicken-cholera."

Control:—A control-rabbit was found dead at 7.50 p.m. the same day (*i.e.*, about $8\frac{1}{4}$ hours after being inoculated). *P.M.*, *Positive.*

Results:

On being fed, at 9 a.m. on the 11th September, the two ferrets appeared dull and feverish. Both drank water freely before touching the meat or porridge and milk given to them, and when they took up the pieces of meat, did not tear at them ravenously, as was their wont before. So they remained for some time. The seat of inoculation showed some special reaction, which in one (the female) subsided gradually, while the condition of the other (male) became worse and worse, till it succumbed on the 18th September.

P.M.—Extensive gangrene round the seat of inoculation; organs abnormal; absence of any micro-organisms in preparations from heart-blood and spleen.

---

* The ferrets referred to in these experiments were sent to the Rabbit Commission by the Government of New Zealand, and were received at Rodd Island on the 31st August. Ferrets are here and there in the Australasian Colonies employed for the destruction of rabbits.

A rabbit inoculated with a small quantity of heart-blood from this ferret, died during the night in consequence of some injuries accidentally received in its hutch.

(ii) With *blood*.

September 11th, 12.50 p.m.

Two fresh ferrets (one male, one female) were inoculated *each* with five platinum-loops full (about 1·50th ccm.) of heart-blood from a rabbit that had died of "chicken-cholera" (inoculation).

Control:—A control-rabbit was found dead at 7.30 a.m. on the 12th September. *P.M., Positive.*

Results:

The seat of inoculation did not show any special reaction. The two ferrets appeared somewhat sluggish at first, but very soon afterwards behaved as before.

(*b*) *Feeding.*

(i) September 12th.

Three fresh ferrets (one male, two females) were fed together upon 30 grammes (about 1·07 oz.) of virulent liver taken from a rabbit newly dead of "chicken-cholera" (inoculation).

Results:

The ferrets did not appear to show any reaction whatever.

(ii) September 18th.

Two fresh ferrets (one male, one female) were fed together upon 45 grammes (about 1·6 oz.) of virulent liver from a rabbit newly dead of "chicken-cholera" (inoculation).

Results:

The ferrets did not show any signs of illness. They remained alive, like the former.

Effect of Desiccation.

In accordance with a desire expressed by the Rabbit Commission at one of its meetings, I have carried out some experiments with a view to testing the influence of desiccation on the microbes of chicken-cholera.

It should be mentioned here that, as more than one observer tells us, the virus of chicken-cholera becomes innocuous by drying up, and that this peculiarity in the life-history of those microbes furnishes an easy and practical means of getting rid of them, wherever they are deposited in poultry-yards. The bacteria of

chicken-cholera are not known to form spores or seeds (as, for instance, the anthrax-bacilli do), by means of which they are able to live under adverse circumstances.

I have to record three series of experiments.

The general plan of procedure was as follows:—A number of silk-threads—of the kind used in surgery—of 1 centimètre in length and $\frac{1}{2}$ to $\frac{5}{8}$ millimètre in thickness, were placed in a sterile cotton-wool-plugged test-tube, and after having been thoroughly moistened with distilled water, were exposed in the steam-steriliser to steam of 100° C. (212° Fahr.) for two hours. The moisture remaining in the silk-threads and in the tube, was got rid of by placing the latter in a copper-box heated up to 100—105° C. (212—221° Fahr.) as long as required.

The virulent material to be tested for its resistance to desiccation consisted, on the one hand, of blood taken from the liver of rabbits which died on inoculation, on the other hand, of fresh broth-cultures derived directly from blood of rabbits newly dead of "chicken-cholera" on inoculation.

The silk-threads referred to above were impregnated with either blood or culture.

In case they were to be impregnated with blood, they were placed on cut-surfaces of the liver, where they remained until they were completely soaked. The livers of all the rabbits used were, I may mention, not otherwise diseased.

In case the threads were to be charged with broth-culture, a small quantity of the latter was placed, by means of a sterile pipette, in a sterile watch-glass, where they remained for some time.

The silk-threads, thus treated either with blood or culture, were then transferred to different places where they could dry up, as will be seen from what follows below. Within certain intervals a silk-thread of both the one and the other description was inoculated into a rabbit each, whereby the virulence or non-virulence of the administered material was to be ascertained. The threads

were in each case deposited in small pouches produced under the skin of the rabbits on the left side of the belly.

I think it necessary to say that every detail of the experiments was managed under due precautions.

1888. *Series I.*

Silk-threads saturated, November 28th, 11 a.m., with fresh liver-blood (containing large numbers of bacteria), and others saturated with fresh broth-culture of the microbes (this culture had been in the thermostat for a day at 39-39½° C., and for another day in the room at a temperature up to 25° C.), were placed on a piece of sterilised brass-wire-gauze in a desiccator over chloride of calcium. This desiccator was placed, immediately after the threads were put in, in the cupboard of a room where the temperature kept pretty even.

The virulence of the material employed (blood and broth-culture) was controlled by means of inoculation of a silk-thread impregnated with either blood or culture into a rabbit each. Both rabbits died promptly of "chicken-cholera," ten and twelve hours, respectively, after inoculation.

Silk-threads were taken out of the desiccator and inoculated into rabbits after 2, 4, 6, 8, 10, 12, 24, 36, 48, 72, 96, 120, 144 hours from the beginning of the experiment.

Within this period of six days, from November 20th to December 5th, the temperature near where the desiccator stood, fluctuated between 21¾° C. and 18° C.

Details about temperatures are given in the following table :—

Date.	Temperatures.
November 29th...	Between 11 a.m. and 11 p.m.: Highest, 21½° C. Lowest, 21° C.
,, 30th...	Between 11 a.m. and 11 p.m.: Highest, 20° C. Lowest, 19° C.
December 1st.....	Between 11 a.m. and 10 p.m.: Highest, 18½° C. Lowest, 18° C.
,, 2nd.....	Between 10 a.m. and 5 p.m.: Highest, 19⅓° C. Lowest, 18° C.
,, 3rd.....	Between 10 a.m. and 10 p.m.: Highest, 20° C. Lowest, 18⅓° C.
,, 4th	Between 9 a.m. and 9 p.m.: Highest, 21¾° C. Lowest, 19° C.
,, 5th.....	At 11 a.m.: 21¾° C.

The result was that the blood which was under the influence of desiccation for three days at the above temperatures, was still able to infect a rabbit, and cause it to perish of "chicken-cholera" (about twenty-one hours after inoculation), whereas after four, five, and six days from the beginning, the desiccated blood had lost its virulence.

On the other hand, the desiccated broth-culture preserved its virulence so far that after two days from the beginning it was still able to kill a rabbit (about twenty-seven hours after inoculation), whereas it was not any longer efficacious when inoculated after three, four, five, and six days' desiccation.

Series II..

Silk-threads saturated, December 7th, 10 a.m., with fresh liver-blood (containing large numbers of bacteria), and others saturated with fresh broth-culture (having been for twenty-four hours in the thermostat at 40° C.—37° C.), were placed on a thin layer of sterilised sandy soil (dry) at the bottom of a shallow basket made of fine brass-wire-netting. (The bottom of this basket had been bent up a little where the sandy soil was put on). The basket was then immediately after placed on a piece of wood, at a distance of about $2\frac{1}{2}$ feet from the ground, in the main enclosure, at a spot which was shaded off by means of a wooden post and of boards, so as to leave the spot only at the south side free and accessible. The basket was sheltered from rain by putting coverings over the top of the boards mentioned.

The virulence of the original material (blood and broth-culture) was tested by inoculating rabbits, one with silk-thread charged with blood, and the other with silk-thread containing broth-culture. Both rabbits died of "chicken-cholera," 10 and 21 hours, respectively, after inoculation.

The effect of the drying-up of the silk-threads was ascertained by inoculating rabbits after 4, 8, 12, 24, 48, 72, 96, 120 hours from the beginning. Within this period, from December 7th, 10.15 a.m., to December 12th, 10.15 a.m., the thermometer in the shaded place registered temperatures of between $20\frac{1}{2}$? C. (lowest) and $29\frac{1}{4}$° C. (highest).

The following Table contains details about the Temperatures, and general remarks on the Atmosphere during that time:—

DATE.	TEMPERATURES IN THE SHADED PLACE.	REMARKS.
December 7th	Between 10.15 a.m. and 2.15 p.m.: Highest 25° C.; lowest $23\frac{3}{4}$° C.	Air dry.
	,, 2.15 p.m. ,, 6.15 p.m.: ,, $24\frac{1}{2}$° C.; ,, 22° C.	Light S. breeze all day.
	,, 6.15 p.m. ,, 10.15 p.m.: ,, 22° C.; ,, 21° C.	
8th	,, 9.40 a.m. ,, 10.15 a.m.: ,, $28\frac{1}{3}$° C.; ,, $27\frac{3}{4}$° C.	Air dry.
	,, 10.15 a.m. ,, 4.30 p.m.: ,, $28\frac{2}{3}$° C.; ,, 25° C.	Very calm.
	,, 4.30 p.m. ,, 9.50 p.m.: ,, 25° C.; ,, $22\frac{1}{4}$° C.	
9th	,, 9.30 a.m. ,, 11.30 a.m.: ,, $21\frac{1}{2}$° C.; ,, $21\frac{1}{4}$° C.	Air dry.
	,, 11.30 a.m. ,, 10 p.m.: ,, $22\frac{3}{4}$° C.; ,, 21° C.	S. breeze all day.
10th	,, 8.15 a.m. ,, 9.45 a.m.: ,, $27\frac{3}{4}$° C.; ,, 26° C.	Air dry, until afternoon, when
	,, 9.45 a.m. ,, 2.15 p.m.: ,, $29\frac{1}{4}$° C.; ,, $25\frac{1}{2}$° C.	thunderstorm set in with rain
	,, 2.15 p.m. ,, 10 p.m.: ,, $25\frac{1}{2}$° C.; ,, 21° C.	for about half an hour.
11th	,, 8.30 a.m. ,, 4 p.m.: ,, $26\frac{1}{4}$° C.; ,, $25\frac{1}{2}$° C.	S. breeze morning.
	,, 4 p.m. ,, 10 p.m.: ,, $25\frac{1}{2}$° C.; ,, $21\frac{1}{4}$° C.	E. breeze afternoon.
		Strong E. wind evening
12th	,, 8.30 a.m. ,, 10.15 a.m.: ,, $22\frac{1}{3}$° C.; ,, $20\frac{1}{2}$° C.	Calm morning.

The result was this:—

The blood thus exposed to desiccation preserved its virulence when inoculated after four, eight, and twelve hours; when inoculated after twenty-four hours and more from the beginning, it had ost its efficacy on rabbits.

The desiccated broth-culture proved virulent only when inoculated four hours after the beginning of the experiment. The rabbit succumbed to "chicken-cholera" twenty-six hours after inoculation. Subsequent inoculations, eight, twelve, and more hours after the beginning, were attended with negative results.

Series III.

Silk-threads saturated, December 7th, 10 a.m., with virulent material (blood and broth-culture) derived from the same sources as the material used in *Series II.*, were placed on sterilised dry sandy soil, which in a thin layer covered the bottom of a small shallow wire-gauze basket, similar to that in *Series II.*

Annotation: Control of virulence as in *Series II.*

At 10.10 a.m., same day, this basket was placed on some available spot on the Island; this spot was accessible to the sun's rays all day long. The bottom of the wire-basket was placed flat on the perfectly dry sandy surface of that spot. This latter was also accessible to the wind or breeze prevailing during the experiment.

The silk-threads remained there from 10.10 a.m. to 6.10 p.m., *i.e.*, for eight hours. Within this period rabbits were inoculated 1, 2, 4, 6, and 8 hours from the time the silk-threads were exposed.

A thermometer was laid on the soil near where the basket with the silk-threads stood. Details about the temperatures at the surface of the soil, during the course of the experiment, are given in the accompanying table.

December 7th:—

10.15 a.m., 45° C.; 10.45 a.m., 50° C.—Sunshine for about ten minutes.

11.15 a.m., 47° C.—Sunshine for about ten minutes since last observation.

11.45 a.m., 42¾° C.—Few minutes sunshine.

12 noon, 35° C.—Cloudy for about twelve of last fifteen minutes.

12.15 p.m., 38° C.—About five minutes sunshine.
12.45 p.m., 36° C.—About five minutes sunshine.
1.10 p.m., 39½° C.—A few minutes sunshine.
1.45 p.m., 42¼° C.—About fifteen minutes sunshine.
2.15 p.m., 38¼° C.—About twenty minutes sunshine.
2.45 p.m., 36¾° C.—Two or three minutes sunshine.
3.15 p.m., 38½° C.—A little sunshine.
3.45 p.m., 33½° C.—Very cloudy.
4.15 p.m., 29½° C.—Very cloudy.
4.45 p.m., 30½° C.—Sunshine for about twenty minutes.
5.15 p.m., 28° C.— } Sun completely obscured.
5.45 p.m., 26° C.— }

From this table there may be seen that the sun was often prevented from making his appearance, by clouds passing by. The day was free from rain, the air was dry, and a southerly breeze was blowing during the time of the experiment.

The result was: the blood exposed to desiccation in this manner proved infectious after one, two, six, and eight hours' exposure (when the experiment was terminated). The rabbits inoculated succumbed to "chicken-cholera" in, respectively, twenty-one, twenty, between thirty and forty, and twenty-eight hours after inoculation. But strange to say, the silk-thread inoculated after four hours' exposure proved inefficacious in so far as the rabbit was still alive, December 17th, ten days after inoculation. It died at about 5.30 p.m., December 18th. *Post-mortem* examination *negative* with regard to "chicken-cholera."

On the other hand, the silk-threads steeped in broth-culture, exposed in exactly the same way, soon lost their efficacy. It was only the first time, after one hour's exposure of the silk-threads, that the inoculation of such a thread proved fatal to a rabbit. It died of undoubted "chicken-cholera" between fifty-nine and sixty-nine hours after inoculation. In all the remaining cases, two and more hours after the beginning of the experiment, the rabbits did not become infected.

From the results thus obtained we learn again that desiccation in general is fatal to the microbes of chicken-cholera. The higher the temperature during the process of desiccation, the less time is required to destroy their virulence.

Desiccation of virulent blood lying on, or impregnating small objects such as the silk-threads used, caused the virus to die off less quickly than is the case with virulent broth-cultures exposed to desiccation under the same circumstances. The reason for this probably is that the superficial portions of the blood drying up, are able to protect the deeper portions for a longer time than is the case with broth-cultures attached to, or saturating small objects, where, by virtue of the composition of the broth, less protection can be afforded to the deeper portions by the superficial ones.

The fact that a virulent broth-culture of the microbes of chicken-cholera very soon ceases to be efficacious when exposed, in a thin layer, to desiccation at summer temperatures such as they exist here, must, in my opinion, to a large extent account for the surviving, now and then, of wild rabbits, which during summer months were given (in shaded hutches) cabbage- or barley-leaves sprinkled with small portions of such a culture, but which were very slow in beginning to eat the infected food, or in finishing it up, so that meanwhile the liquid spread on it was enabled to dry up.

Effect of Putrefaction.

It is ascertained that the bacteria of chicken-cholera, when kept together with other micro-organisms, as in contaminated cultures, are sometimes able to retain their vitality, and power of infecting, for a considerable time, up to three months.*

For my own part, I have tested how long chicken-cholera bacteria would remain active in rabbit-blood which, containing

*Kitt, Wert und Unwert der Schutzimpfungen gegen Tierseuchen Berlin, 1886, p. 55.

the organisms in their full virulence, was allowed to putrefy at a moderate temperature. From the obtained results it follows that in putrefying or putrid blood of the above kind, they may be found still efficacious after weeks.

1888.

At the examination, on the 2nd September, of a rabbit about four days after its death from "chicken-cholera," in one of the burrows in the large enclosure on the Island, the coagulated blood of the right ventricle of the heart was removed and placed in a small clean, not sterilised, glass-flask which was stoppered and put aside in the laboratory. On microscopical examination on the date mentioned, only the microbes of chicken-cholera were present.

(i) September 3rd, 11.30 a.m.

A half-grown rabbit, *inoculated* with a small platinum-loop full of this blood (not yet putrid), was found dead at 7 a.m. on the 3rd September. *P.M.*, *Positive.*

(ii) September 10th, 5 p.m.

A rabbit, *inoculated* with about the same quantity of the blood (now putrid), was found dead at 8.25 a.m. on the 11th September, having died between 7.15 a.m. and that time (*i.e.*, between $14\frac{1}{4}$ and $15\frac{1}{2}$ hours after being inoculated). *P.M.*, *Positive.*

(iii) September 17th, 2.10 p.m.

A rabbit, *inoculated* with about the same quantity of the blood (putrid), was found dead at 7.40 a.m. on the 18th September. *P.M.*, *Positive.*

(iv) September 20th, 10.40 a.m.

A rabbit, *inoculated* with about the same quantity of the blood (putrid), was found dead at 5 p.m. on the 22nd September, having died between 1.50 p.m. and that time (*i.e.*, between 51 and 54 hours after inoculation). *P.M.*, *Positive.*

(v) September 24th, 11.10 a.m.

A rabbit, *inoculated* with about the same quantity of blood, remained alive after this treatment.

APPENDIX I.

Note on the Transition of Pathogenic Bacteria from the mother to the fœtus.

Several pathogenic micro-organisms, especially those which cause lesions of the vascular system (hæmorrhages, thromboses) in the different organs [*e.g. Bacillus anthracis*; *Streptococcus septicus* (Flügge)], are known to be able to pass from the mother to the fœtus. Fraenkel's *pneumococcus* is also capable of so doing; in tuberculosis a passage of the bacillus through the *placenta* appears to exist, but rarely occurs, it is said. In typhoid fever the possibility of a transmission of the bacillus of this disease from the mother to the child has lately been established (J. C. Eberth).*

With regard to chicken-cholera, Marchiafava and Celli found the bacteria of this disease in the fœtus of a guinea-pig which had been successfully infected with those microbes.

On pp. 569, 570, I have given notice of an experiment on guinea-pigs, which were fed on cabbage-leaves sprinkled with virulent chicken-cholera microbes. One of two guinea-pigs which subsequently died from "chicken-cholera," namely a full-grown doe, had in the right uterus a fœtus measuring 53 mm. in a straight line from the vertex of the head to the root of the tail. I will repeat here that the hæmorrhage in the small intestine of the mother animal was less considerable and less marked than in the case of the other younger doe which also died.

Samples of heart-blood and of liver-substance were carefully derived from the above fœtus, and cover-glass preparations made. These were fixed, stained, and examined with homogeneous im-

* *Centralblatt für Bakteriologie und Parasitenkunde.* Band V., No. 19, 1889, pp. 643, 644. See also E. Malvoz, Le passage des micro-organisms au fœtus. Revue critique. *Annales de l'Institut Pasteur.* Tome III., No. 4, 1889, pp. 188-193.

mersion objective, as usual, but there was neither a sign of chicken-cholera bacteria nor of any others. By that, however, it cannot be asserted that the blood of the fœtus must have been absolutely free from such bacteria, because culture-experiments, which would have been decisive, were not carried out.

In rabbits also, the results obtained from a few similar microscopical examinations were negative. Examined were (1) heart-blood of two out of seven fully-developed fœtuses which had been dropped by a doe dead from inoculated "chicken-cholera." In this case, however, the young ones might have been born soon after the inoculation of the mother-rabbit took place. (2) liver-substance of two of several fœtuses contained in the uterus of a doe dead after inoculation; this doe was in the beginning of gestation. (3) liver-substance of one of a few fœtuses taken from the uterus of a doe dead after inoculation; this doe was in about the end of the second week of gestation.

These negative findings, I confess, cannot claim an absolute value from want, again, of any culture-experiments in gelatine being carried out with samples of the fœtal organs; yet they are quite in agreement with the fact that "chicken-cholera" in rabbits, at least in those with which I had to do, presented itself as a rapidly killing septicæmia, in which, if we except the lungs, any visible lesions of the blood-vessels are rarely found.

APPENDIX II.

Remarks on Gamaleia's article "A Contribution to the Etiology of Chicken-cholera, with Notes on the Question of Protective Vaccination."*

In this article Gamaleïa states as the result of direct experiments, which he describes, that microbes of chicken-cholera constantly

* Zur Aetiologie der Hühnercholera. Nebst einigen Bemerkungen über die Schutzimpfungsfrage. Von Dr. N. Gamaleïa, Vicedirector der bakteriologischen Station in Odessa. *Centralblatt für Bakteriologie und Parasitenkunde.* Band IV., 1888, pp. 161-168.

inhabit the normal intestinal canal of pigeons, perhaps also of other birds, similarly as the septic vibrio (the bacillus of malignant œdema) is always present in mammals. In such state the microbes are not virulent enough to do any harm to their host, or to other poultry into which they are inoculated; they are, however, able to cause disease and death in the case of very susceptible, though healthy, animals, namely rabbits and "Ziesel" (also rodents, Genus *Spermophilus*). Transmitted through the body of a rabbit or a "Ziesel," they attain such a strength that they are able to kill pigeons and fowls; on the other hand, fowls can be rendered immune against deadly infection by chicken-cholera bacteria, by means of the inoculation of certain doses of the above virus (passed through rabbits, *e.g.*, from the intestines of healthy pigeons). With regard to the important question: under what conditions the originally harmless bacteria exhibit their dreaded epidemic virulence, Gamaleïa favours the view of the "removal from the intestinal canal of all mesoderm-phagocytes which must be engaged in the digesting of large quantities of the introduced saprophytes." He proposes to strike out the altogether inappropriate designation "bacteria of chicken-cholera," and to substitute the more scientific name "bird-septicæmia." The name for the concerning microbes shall be *coccobacillus avicidus*, which must be assigned to the entosaprophytes which are also facultative parasites.

After having taken information of Gamaleïa's interesting paper I wished to know whether I should succeed in proving the occurrence of attenuated forms of the bacteria of chicken-cholera in normal pigeons, on Australian soil. The tests were made on wild rabbits which throughout were known to me as highly accessible to virulent "chicken-cholera." The results, however, did so far not confirm Gamaleïa's statement; they were all negative, as shown by the following list of experiments. At the end is mentioned the examination of a chick, with the same result.

1888.

(1) September 27th, 3.50 p.m.

A healthy pigeon* was killed by chloroform-narcosis. The contents of the small and large intestines, and part of the contents of the stomach (the latter containing green food) were taken under antiseptic precautions, placed together in a test-tube, and mixed and shaken with about 10 ccm. of sterile distinctly alkaline rabbit-broth. This tube was for a while put in a water-bath at 37° C.

Of this mixture, 1 ccm. was injected subcutaneously into each of two rabbits by means of a sterilised pointed glass-tube.

Results:

(a) One rabbit was found dead at 6 p.m., September 28th. *P.M., Negative.*

A healthy pigeon, inoculated at 10.30 a.m., September 29th, with a platinum-loop full of heart-blood from this rabbit, remained alive and well. A half-grown rabbit, inoculated at 10.45 a.m., same date, with one platinum-loop full, and a full-grown rabbit, inoculated at 11.30 a.m., same day, with five platinum-loops full of heart-blood of the same rabbit, were both alive at 4 p.m., October 8th, when they were removed from their hutch.

(b) The other was still alive at 4 p.m., October 8th, when it was turned loose among others.

(2) October 3rd, 10.40 a.m.

A healthy pigeon was killed by chloroform-narcosis. The contents of the intestines were collected under antiseptic precautions, mixed and shaken with sterile rabbit-broth (as above) in a test-tube, and warmed as before.

Of this mixture injections were made (analogously to the first experiment) into two rabbits. At 11.10 a.m.,

A half-grown rabbit received $\frac{1}{2}$ ccm. of the mixture,

A full-grown rabbit received 1 ccm. of the mixture.

Results:

Both of these rabbits were alive at 9 a.m., October 11th, when they were removed from their hutch.

---

* All the pigeons mentioned here were among a consignment of twelve purchased at the Sydney Markets.

(3) November 23rd, 5 p.m.

One of two pigeons, taken out of a consignment of twelve obtained from the Sydney markets, on 8th November—the remaining ten were with two others placed in an aviary, where they were used for another experiment—was killed by chloroform-narcosis. The contents of the intestines, including a portion of the contents of the stomach, were derived under proper precautions, and thoroughly mixed and shaken with about 10 ccm. of sterile distinctly alkaline rabbit-broth in a test-tube.

(*a*) Of this mixture, 1 ccm. each was injected into a full-grown and a half-grown rabbit soon afterwards.

Results:

The half-grown rabbit observed lying dead at 7.30 a.m., November 29th. *P.M.*, *Negative* (both as regards appearance of organs and microscopical examination of liver-blood).

The full-grown rabbit being still alive at 10 a.m., December 2nd, was removed from its hutch.

(*b*) The above mixture was, after the 2 ccm. had been taken out, put into a thermostat where it remained for about 24 hours at about 39° C.

November 24th, 5.30 p.m.

A full-grown rabbit received $\frac{1}{4}$ ccm. = 4 minims,

A half-grown rabbit received $\frac{1}{8}$ ccm. = 2 minims

of the culture obtained from the mixture.

Results:

The full-grown rabbit died at 11.30 a.m., November 30th. *P.M.*, *Negative.*

The half-grown rabbit observed to die at 7.30 a.m., November 25th. *P.M.*, *Negative.*

(4) December 11th, noon.

The remaining of the two pigeons was killed by chloroform-narcosis. About half the contents of the intestines, including part of the contents of the stomach, were transferred to a spacious test-tube containing about 15 ccm. of sterile rabbit-broth which was of a distinctly alkaline reaction.

The mixture after being well-shaken showed still a slightly alkaline reaction. The tube was at once placed in the thermostat at 38° C. to 38$\frac{3}{4}$° C., for about twenty-four hours.

December 12th, 1 p.m.—Of the culture obtained (showing now a slightly acid reaction), a very vigorous full-grown doe received (subcutaneously) 1 ccm.; a rabbit not quite full-grown, ½ ccm.

Results:

Both rabbits were alive for a considerable time.

They both died in succession, the following month (January, 1889), but not from "chicken-cholera," or anything similar.

(5) September 14th, 11.30 a.m.

A half-grown rabbit was inoculated with a portion of the contents of the intestines of a young chick sent to me the previous day (dead) from Burwood, near Sydney. (The mortality amongst chickens there had been very great that year, according to information.) The rabbit died between 11 a.m. and 12.30 p.m., September 15th, but on examination it was found that the cause of death could not have been an infection by chicken-cholera microbes. (Another half-grown rabbit was inoculated, at the above date, with heart-blood from the same chick; it also died about a day afterwards, the result of the autopsy likewise excluding "chicken-cholera.")*

---

* In connection with the above subject it may not be uninteresting to mention that up to the present, chicken-cholera, so devastating and dreaded a disease in other countries, has not been proved to exist in Australasia. I mean, of course, the typical disease with its well-characterised microbes, and not other disorders met with in poultry, where, misled by certain suspicious symptoms, one may think of the true cholera (poultry-typhoid). The Rabbit Commission received specimens of dead fowls or blood from such, mostly from New South Wales, twice from Victoria, and once from New Zealand, in all nine cases. They were examined by me; inoculations were made into fowls (six times), mice (once), rabbits (once), besides mostly examining microscopically the blood, or obtaining in nutrient gelatine colonies of the bacteria present in the suspicious specimens. However, the results showed that bacteria of chicken-cholera were not there. It is to be regretted that at the time of these examinations, rabbits which are susceptible to attenuated "chicken-cholera" (according to Gamaleïa), were not at my disposal, except in one case [(5) above]. Further researches in this direction may ultimately lead to positive results.

TABLE I.

TABLE of Temperatures, both in air (shaded), and underground (bottom of special small burrow or trench without entrance ; in other respects similar to the proper burrows), in enclosure. General remarks on the weather are found in the last column.

Date. 1888.	Temperatures (°C.). In air (shaded), or underground.	at 7 a.m.	11 a.m.	3 p.m.	7 p.m.	10.30 p.m.	General remarks on the weather.
November 7th.	Air.....			19½	17½	16½	Noon : cool S. breeze. Calm in afternoon, until night.
	Underground			21¼	20½	19¾	
,, 8th.	Air.....	16	19½	19	16½	16¼	Few light showers during last night. 7.30 a.m.: cold S. breeze; fine. 11 a.m.: light breeze; fine. Light S. breeze all afternoon. Evening calm.
	Underground	19½	20	20¾	20	19½	
,, 9th.	Air.....	17¾	24½	20½	17¾	17	Calm all day. Light E. breeze afternoon.
	Underground	19¼	20	21¼	20¾	19¾	
,, 10th.	Air.....	21¾	25¼	22½	20½	18½	7.30 a.m. : calm. Light E. breeze morning. Slight showers 1.30 p.m. to 2.10 p.m. Calm afternoon and evening.
	Underground	19½	21	21¼	21	21	
,, 11th.	Air.....	18¾	24½	23	19¼	17½	Calm morning. Strong E. wind afternoon. Calm evening.
	Underground	19¼	21¼	21½	21½	21¼	

TABLE I.—*(Continued).*

Date. 1888.	Temperatures (°C.). In air (shaded), or underground.	at 7 a.m.	at 11 a.m.	at 3 p.m.	at 7 p.m.	at 10.30 p.m.	General remarks on the weather.
Novemb. 12th.	Air.....	21	25¼	25	24	18¼	Calm morning. E. wind most of afternoon. Calm evening.
	Underground	20¼	21½	22	22	21½	
,, 13th.	Air.....	23½	28	29½	25	18¼	Strong N.W. wind blowing all morning and most of afternoon. Calm evening.
	Underground	20¾	21½	22	24	22	
,, 14th.	Air.....	21½	27¾	26	22	19¾	Light E. breeze morning. Afternoon and evening calm.
	Underground	21½	21½	23¼	22¼	21¼	
,, 15th.	Air.....	26¼	28¼	32½	21¾	20	N.W. wind all morning and afternoon.
	Underground	21½	21¾	24	22¾	22¼	
,, 16th.	Air.....	19¾	19	18¼	16	17¼	Calm, dull, sky overcast. Few drops of rain 6.30 a.m. 9.35 to 10 a.m.: light showers, calm. Noon: light E. breeze, dull. 3 p.m.: calm, dull. Light drizzling rain from 6 p.m. to about 7.15 p.m. Thereafter calm, sky cloudy.
	Underground	21¾	21½	21¾	21¼	20½	
,, 17th.	Air.....	19	21½	22½	20½	20	Morning: sky overcast, light E. breeze. Afternoon: E. wind. Evening calm.
	Underground	20½	20¾	21¼	21	20¾	
,, 18th.	Air.....	19½		22¼	18⅔	18	Light S.E. breeze until 11 a.m. S E. wind until night. Calm night.
	Underground	19½		20¾	21¾	20¼	
,, 19th.	Air.....	17½	19⅓	19½	17	15¼	Calm and dull all day.
	Underground	20½	21	21½	21½	20¾	

TABLE I.—*(Continued).*

Novemb. 20th.	Air.....	$17\frac{3}{4}$	$20\frac{3}{4}$	21	$18\frac{1}{3}$	18	Calm, sky overcast until noon. Bright—N.E. breeze—afternoon. Calm night.
	Underground	$20\frac{1}{4}$	21	22	$21\frac{3}{4}$	21	
,, 21st.	Air.....	$18\frac{3}{4}$	26	$23\frac{1}{4}$	$19\frac{1}{4}$	$17\frac{1}{4}$	7 a.m.: calm, sky overcast. Bright, with N.E. wind, morning and afternoon. Calm night.
	Underground	$20\frac{1}{4}$	$21\frac{1}{2}$	$23\frac{1}{2}$	22	21	
,, 22nd.	Air.....	19	27	$26\frac{1}{4}$	$21\frac{1}{4}$	$18\frac{1}{4}$	7 a.m.: calm. N.E. breeze morning and afternoon. Calm night, sky overcast.
	Underground	$19\frac{1}{2}$	23	25	$23\frac{1}{2}$	$23\frac{1}{2}$	
,, 23rd.	Air.....	18	$29\frac{1}{2}$	$29\frac{1}{4}$	24	$21\frac{1}{4}$	Bright — N.E. breeze — morning and afternoon. Night calm, sky overcast.
	Underground	$20\frac{1}{4}$	23	$22\frac{1}{2}$	22	22	
,, 24th.	Air.....	$20\frac{1}{4}$	$32\frac{1}{2}$*	$31\frac{1}{4}$	$22\frac{1}{4}$	$20\frac{1}{2}$	7 a.m.: calm. 8 a.m.: extraordinarily muggy and misty till 5.30 p.m., when strong S. wind till 7.30 p.m. Night calm, sky overcast.
	Underground	$20\frac{1}{2}$	23	$25\frac{1}{4}$	25	$22\frac{3}{4}$	
,, 25th.	Air.....	$19\frac{3}{4}$	$21\frac{1}{4}$	21	$18\frac{1}{2}$	$16\frac{1}{4}$	Bright—varying N.E. and S.W. breezes—morning and afternoon. Night calm, sky overcast.
	Underground	21	$22\frac{3}{4}$	$23\frac{1}{2}$	$23\frac{1}{4}$	$23\frac{1}{4}$	
,, 26th.	Air.....	19	$21\frac{3}{4}$	$24\frac{1}{3}$	$20\frac{1}{2}$	$20\frac{1}{4}$	Calm and dull all morning. Bright with N.E. breeze, afternoon. Night calm, sky overcast.
	Underground	23	$23\frac{3}{4}$	$23\frac{3}{4}$	23	$22\frac{1}{2}$	
,. 27th.	Air.....	$22\frac{3}{4}$	$27\frac{1}{4}$	$26\frac{1}{3}$	$23\frac{1}{4}$	22	Morning: bright; N.E. breeze. Afternoon dull and calm. Night: sky overcast; rain, 9·30 to 10 p.m.
	Underground	$22\frac{1}{4}$	$24\frac{1}{4}$	$25\frac{3}{4}$	$24\frac{3}{4}$	$24\frac{1}{4}$	
,, 28th.	Air.....	23	$28\frac{3}{4}$	$27\frac{3}{4}$	$22\frac{1}{2}$	$20\frac{1}{2}$	7 a.m.: calm and dull. Later: light N.E. breeze. 4 p.m.: gloomy; S. and S.W. winds. 7 p.m. to 10.30 p.m.: showery.
	Underground	23	$24\frac{1}{2}$	26	$24\frac{1}{4}$	$23\frac{1}{2}$	
,, 29th.	Air.....	$21\frac{1}{4}$	$22\frac{1}{4}$	22	$20\frac{3}{4}$		Dull, with S.W. breeze, all morning. Dull, calm evening. Night: raining.
	Underground	$22\frac{1}{4}$	$22\frac{1}{2}$	$22\frac{1}{2}$	$21\frac{3}{4}$		

* 11.45 a.m., $34\frac{1}{4}$; 1 p.m., $36\frac{3}{4}$ (greatest heat).

TABLE II.

TABLE of Temperatures, of air (shaded), of the surface of the soil (not shaded), and underground (bottom of specially made small burrow, as in Table I.), in enclosure. In the last column may be found general remarks on the weather during the course of the experiment.

Date. 1889.	Temperatures (°C.) Air (shaded), Surface of soil, Underground.	At 7 a.m.	11 a.m.	3 p.m.	7 p.m.	10.30 p.m.	General remarks on the weather.
February 12th.	Air			28	22¾	21¼	Afternoon: bright, N.E. wind, air dry. Night: calm.
	Surface of soil			32	22½	21	
	Underground			26	26	24	
,, 13th.	Air	19	29	29⅓	24¾	22½	7 a.m.: intensely foggy, air moist. 11 a.m.: bright, calm. 3 p.m.: bright, N.E. wind, air dry. 10.30 p.m.: calm, clear.
	Surface of soil	21	36½	33¼	24	22½	
	Underground	26	26	24	23¾	24	
,, 14th.	Air	21	31⅓	26	23¼	22½	Morning: bright, close, moist. 11 a.m.: N.E. breeze. Afternoon: S. wind, sky overcast. Night: S. breeze, clear.
	Surface of soil	22½	38½	34½	24¾	23	
	Underground	22½	23	23⅜	24	24½	
,, 15th.	Air	23	28	26	23¾	22¾	7 a.m.: dull, calm. Later on till evening: N.E. breeze, dull. 7 p.m.: calm, dull. 9 p.m.: W. breeze, thunder and lightning without rain.
	Surface of soil	24½	38¼	31½	24¾	23	
	Underground	23¼	23½	24½	24¼	24¼	
,, 16th.	Air	23	25	24½	22	22	7 a.m.: S. and S.E. winds, cloudy. Afternoon: S. and S.E. breezes, cloudy.
	Surface of soil	24	32¾	29½	23¼	22¾	
	Underground	23½	24	24¼	24¼	24	
,, 17th.	Air	23½	28	24⅜	22½	21¾	Morning: bright. 3 p.m.: dull. Night: sky overcast. N.E. breeze all day.
	Surface of soil	25½	38¼	27¼	23¼	22¾	
	Underground	24	24¼	24¼	24¼	23¾	

TABLE II.—*(Continued).*

February 18th.	Air.....	21½	30	30	24½	23	7 a.m.: calm, dull. Morning and evening: N.E. breeze, bright, but close. Night: calm, clear.
	Surface of soil	23	40	36	25	23½	
	Underground	23½	24	25	25	25¼	
,, 19th.	Air.....	22	22	21½	20¾	20½	7 a.m.: N.E. breeze, misty. Later: S. and S.E. breezes, sky overcast.
	Surface of soil	22½	24¾	24¼	21½	21¼	
	Underground	24	24	24	23½	23½	
,, 20th.	Air.....	18¾	21¾	22¾	21	21	All day: calm, sky overcast.
	Surface of soil	20½	23½	26	21¾	21½	
	Underground	23	23½	23½	23¼	23¼	
,, 21st.	Air	19½	25	27½	24	22¾	7 a.m.: calm, hazy. Noon: N.E. wind; bright, clear. Night: calm, sky overcast.
	Surface of soil	21¾	28¼	34½	24	23	
	Underground	23	23	24	24¼	24¼	
,, 22nd.	Air.....	22	34¼	24	23½	22	All day: very calm, close, and oppressive. Afternoon: about 1½ hour's rain, with thunder. Night: calm, sky overcast.
	Surface of soil	24	41	24½	23½	22	
	Underground	23½	24	24⅜	24¾	24¾	
,, 23rd.	Air.....	22	22½	27	22¾	22	7 a.m.: N.E. breeze, sky overcast. 11 a.m.: light S. breeze. 3 p.m.: light N.E. breeze, bright. 7 p.m.: N.E. breeze, sky overcast. 10.30 p.m.: calm, sky overcast.
	Surface of soil	22	24	30½	23	22	
	Underground	24	23½	24·	24¼	24¼	
,, 24th.	Air	22½	29¼	32¼	28½	25¼	7 a.m.: calm, sky overcast. Later: N.E. breeze, bright. Sultry all day and night.
	Surface of soil	23¼	39	40	27½	24¼	
	Underground	23½	24	25	25¼	25¼	
,, 25th.	Air.....	22¼	24¾	25	22	21¾	Morning and evening: S. and S.E. breezes, bright. Night: S. breeze, sky overcast.
	Surface of soil	23	34½	32¼	23¼	23	
	Underground	24½	24½	25	25	24¾	

TABLE II.—*(Continued).*

Date. 1889.	Temperatures (°C.)						General remarks on the weather.
	Air (shaded), Surface of soil, Underground.	At 7 a.m.	At 11 a.m.	At 3 p.m.	At 7 p.m.	At 10.30 p.m.	
February 26th.	Air	21¼	28	27¾	25	24½	7 a.m.: calm. 11 a.m.: N.E. breeze. 3 p.m.: strong N.E. wind, bright all day. 7 p.m.: light N.E. wind, threatening. Night: some thunder and lightning, close.
	Surface of soil	22½	35½	33¾	25½	24¾	
	Underground	24	24	25	25	25¼	
,, 27th.	Air	23½	33	38	29¼	27½	Morning: calm, oppressive. Later: N., N.W. and W. winds. Afternoon and night: calm, oppressive, a few drops of rain.
	Surface of soil	24½	36½	37¼	28½	26½	
	Underground	24¼	25	26	26	26	
,, 28th.	Air	20¾	22¼	21	20½	19½	7 a.m.: S. breeze, drizzling rain. Later: S. wind, cloudy.
	Surface of soil	21½	27	24	21½	21	
	Underground	24	24¾	25	25	25½	
March 1st.	Air	19¾	21¾	21	20	19	Morning: S. and S.E. breezes. Afternoon: calm. 7 p.m: S. breeze. 10.30 p.m.: rain. Sky overcast all day.
	Surface of soil	20	24	23½	21½	19½	
	Underground	23	23½	23½	23¼	23¼	
,, 2nd.	Air	19	24¾	24¾	22¼	22	7 a.m.: calm, sky overcast. Later: N.E. breeze, bright. 7 p.m.: few drops of rain. 10.30 p.m.: calm, damp.
	Surface of soil	20	29½	28½	22	21⅓	
	Underground	22¾	23	23¾	24	23⅓	
,, 3rd.	Air	21	25¾	27½	23¼	21½	Morning: calm, bright. Afternoon: N.E. breeze, bright. Night: calm, sky overcast.
	Surface of soil	21¼	32	33⅔	23¾	21⅓	
	Underground	23	23½	24½	24½	24	
,, 4th.	Air	20½	27¼	28½	24	23¼	7 a.m.: calm, intensely hazy. Later: N.E. wind, bright. Night: calm, sky overcast.
	Surface of soil	22⅔	33¾	34	23½	23½	
	Underground	23½	24	25	25	24¾	
,, 5th.	Air	22	29¾	28½			7 a.m.: calm, bright. Later: strong N.E. wind, bright.
	Surface of soil	22¼	40	34⅓			
	Underground	24	24⅓	25			

TABLE III.

Table showing Results of the Inoculation Series.

Series.	Distinctive number of rabbit.	Inoculated from	Inoculation, Date and time.	Time from death of rabbit in the preceding Series. From time of death.	Time from death of rabbit in the preceding Series. From time of being found dead.	When seen to die, or when found dead.	Time from inoculation to death. When seen to die.	Time from inoculation to death. When found dead. Between	Temperature of body when found dead (centigrade).	Remarks.
			1888.	H.M.	H.M.		H.M.	H.M. and D.M.		
I	1	A pigeon that died of chicken-cholera.	Oct. 4th, 11 30 a.m.			Oct. 4th, 9.40 p.m.		9.25 and 10.10		
	2		Oct. 4th, 11.35 a.m.			Oct. 5th, 5.40 a.m.		12.25 ,, 13.5	39·45	
II	3	The first rabbit of the preceding Series.	Oct. 4th, 10·15 p.m.		35	Oct. 5th, 1.5 p.m.	13.20	14.10 ,, 14.50		
	4		Oct. 4th. 16.10 p.m.		36	Oct. 5th, 4.36 p.m.				
III	5	,,	Oct. 5th, 2.15 p.m.		1,10	Oct. 6th, 5 a.m.		12.6 ,, 14.50		
	6		Oct. 5th, 2.5 p.m.		1.6	Oct. 6th, 7.50 a.m.		15.40 ,, 17.45		
IV	7	,,	Oct. 6th, 5.35 a.m.		35	Oct. 6th, 6.50 p.m.	13.15			
	8		Oct. 6th, 5.25 a.m.		25	Oct. 6th, 8.40 p.m.	15.15			
V	9	,,	Oct. 6th, 8.15 p.m.	1.25		Oct. 7th, 9.10 a.m.	12.55			
	10		Oct. 6th, 8.5 p.m.	1.15		Oct. 7th, 9.5 a.m.	13.0			
VI	11	,,	Oct. 7th, 10.47 a.m.	1.37		Oct. 8th, 12.25 a.m.	13.38			
	12		Oct. 7th, 10.40 a.m.	1.30		Oct. 8th, 12.42 a.m.	14.2			
VII	13	,,	Oct. 8th, 1.16 a.m.	51		Oct. 8th, 4 p.m.	14.44			
	14		Oct. 8th, 1.30 a.m.	1.5		Oct. 8th, 8.55 p.m.	19.25			
VIII	15	,,	Oct. 8th, 4.57 p.m.	57		Oct. 9th, 6.55 a.m.	19.22	13.8 ,, 13.58		
	19		Oct. 8th, 5.5 p.m.	1.5		Oct. 9th, 9.27 a.m.				
IX	17	,,	Oct. 9th, 8.15 a.m.		1.20	Oct. 9th, 7.20 p.m.	11.5			No. 17.—Somewhat smaller specimen than usual. Seat of inoculation showing larger area of hæmorrhagic gelatinous œdema than usual. In the liver a few coccidium abscesses.
	19		Oct. 9th, 8.22 a.m.		1.27	Oct. 9th, 11.31 p.m.	15.9			
X	19	,,	Oct. 9th, 8.38 p.m.	1.18		Oct. 10th, 9.10 a.m.		11.47 ,, 12.32	37·1	No. 19.—A few coccidium abscesses in the liver.
	20		Oct. 9th, 8.30 p.m.	1.10		Oct. 10th, 11.45 a.m.		14.45 ,, 15.15		
XI	21	,,	Oct. 10th, 16.29 a.m.		1.19	Oct. 10th, 7.55 p.m.	14.21	7.29 ,, 9.29	38·0	No. 21.—Specimen only about three-quarters grown; rather thin. Few coccidium abscesses in the liver.
	22		Oct. 10th, 10.34 a.m.		1.24	Oct. 11th, 12.55 a.m.				
XII	23	,,	Oct. 10th, 8.55 p.m.		1.0	Oct. 11th, 8.25 a.m.		10.5 ,, 11 30	38·8	
	24		Oct. 10th, 9.7 p.m.		1.12	Oct. 11th, 1.35 p.m.		16.3 ,, 16.28	40·3	
XIII	25	,,	Oct. 11th, 16.9 a.m.		1.43	Oct. 11th, 11.43 p.m.	13.35			
	26		Oct. 11th, 9.58 a.m.		1.33	Oct. 11th, 12.47 a.m.		14 42 ,, 14.49	38·6	
XIV	27	,,	Oct. 12th, 12.46 a.m.	1.3		Oct. 12th, 6.32 p.m.		17.37 ,, 17.49		
	28		Oct. 12th, 12.39 a.m.	58		Oct. 12th, 9.13 p.m.		19.41 ,, 20.34	40·45	
XV	29	,,	Oct. 12th, 7.40 p.m.		1.9	Oct. 13th, 5.57 a.m.	10.17			No. 29.—A doe which had dropped seven fully-developed young, some of which were found alive the next morning.
	30		Oct. 12th, 7.53 p.m.		1.21	Oct. 13th, 9.15 a.m.		12.37 ,, 13.22		
XVI	31	,,	Oct. 13th, 9.15 a.m.	2.13		Oct. 13th, 11.50 p.m.	15.41			No. 31.—A doe in the beginning of gestation.
	32		Oct. 13th, 8.7 a.m.	2.10		Oct. 14th, 12.45 a.m.		15.48 ,, 16.38		
XVII	33	,,	Oct. 14th, 1.10 a.m.	1.14		Oct. 14th, 11.37 a.m.		10.10 ,, 10.27		No. 34.—A doe, about which something more on page 513.
	34		Oct. 14th, 1.20 a.m.	1.24		Oct. 16th, 1.55 a.m.		45.26 ,, 48.35	37·4	
XVIII	35	,,	Oct 14th, 12.54 p.m.		1.17	Oct. 15th, 12.30 a.m.		11.20 ,, 11.45	41·7	
	36		Oct. 14th, 12.45 p.m.		1.8	Oct. 15th, 1.55 a.m.	13.10			
XIX	37	,,	Oct. 15th 1.41 a.m.		1.11	Oct. 15th, 4.38 p.m.	14.57			No. 38.—A doe in about the end of the second week of gestation.
	38		Oct. 15th, 1.32 a.m.		1.2	Oct. 15th, 8.50 p.m.		16.33 ,, 17.18		
XX	39	,,	Oct. 15th, 5.45 p.m	1.7		Oct. 16th, 7.5 a.m.		9.25 ,, 13.20	35·6	
	40		Oct. 15th, 5.38 p.m.	1.6		Oct. 16th, 7.20 a.m.	13.42			

TABLE IV.

TABLE showing results of inoculation of fowls and pigeons with the virus of chicken-cholera, taken from certain cases out of the Inoculation Series of Rabbits (Table III).

Series.	Fowl or pigeon.	Inoculated from virulent blood of rabbit of	Inoculation. Date and time.	Inoculation. Time from death of rabbit to inoculation of fowl or pigeon. From time of death.	Inoculation. Time from death of rabbit to inoculation of fowl or pigeon. From time of being found dead.	When seen to die, or when found dead.	Time from inoculation to death. When seen to die.	Time from inoculation to death. When found dead. Between	Remarks.
			1888.	H. M.	H. M.		H. M.	H. M. H. M.	
I	Fowl	Inoculation Series I.	Oct. 16th, 2.40 a.m.		45*	Oct. 16th, 11.30 p.m.	20.50	17.25 and 18.30	*Body when found was cold. Fowl must have died between 12 h. 30 m. and 16 h. 45 m. after inoculation.
	Pigeon		,, 2.50 a.m.		55	,, 9.20 p.m.			
II	Fowl	Inoculation Series V.	Oct. 7th, 11 a.m.	1.50		Oct. 8th, 7.35 a.m.		14 30 ,, 20.35	
	Pigeon		,, 10.52 a.m.	1.42		,, 7.35 a.m.		14.38 ,, 20.43	
III	Fowl	Inoculation Series X.	Oct. 10th, 10.45 a.m.		1.35†	Oct. 11th, 8.25 a.m.		20.15 ,, 21.40	†Temperature when found dead 37·1° C.
	Pigeon		,, 10.50 a.m.		1.40	,, 6.50 a.m.		14.15 ,, 20.0	This fowl had laid an egg between 7 a.m. and 8.25 a.m., which appeared perfectly normal as regards both the exterior and interior. On microscopical examination of the yolk, micro-organisms could not be detected.
IV	Fowl	Inoculation Series XV.	Oct. 13th, 8.25 a.m.	2.28		Oct. 14th, 8.30 a.m.		23.20 ,, 24.5	
	Pigeon		,, 8.33 a.m.	2.36		,, 13th, 11.10 p.m.		13.57 ,, 14.37	
V	Fowl	Inoculation Series XX.	Oct. 16th, 10. 6 a.m.		3 .0‡	Oct. 17th, 5.45 a.m.		17.20 ,, 19.40	‡Temperature when found dead 35·6° C.
	Pigeon		,, 10.10 a.m.		3 .5	,, 5.45 a.m.		17.15 ,, 19.35	

TABLE V.

TABLE showing Body Temperatures of certain of the Rabbits used in the Inoculation Series.

Number	Corresponding Series and Number in Table III.		Temperatures (Centigrade).																									Remarks.
			Hours after inoculation.																									
	Series.	Number.	At time of inoculation.	2	2½	3	4	4½	5	5½	6	6½	7	7½	8	8½	9	9½	10	10½	11	11½	12	12½	13	13½	14	14½	15	
1	I	1		39·8			39·9				40·2				40·7				†										
2	,,	2	39·9	39·4			40·7				40·7				40·0				40·95										† Between 12h. 25m. and 18h. 6m. after inoculation.
3	III	5	39·85	39·7				40·0					40·0				4·8						41·0						† Between 12h. and 14h. 50m. after inoculation.
4	,,	6	40·2	40·1				39·55					40·1				40·4						40·25						† Between 15h. 40m. and 17h. 45m. after inoculation.
5	IV	7	40·0					40·35				40·6				41·4				41·47					39·6†				
6	,,	8	39·2					38·8				39·1					39·4				39·25					40·6		39·67†	
7	VI	11	38·9	39·07			39·3				39·0				39·4				40·26					40·8		39·6†			
8	,,	12	39·1	39·6			39·8				40·05				40·2					41·0				41·6			40·2†		
9	IX	17	38·85			39·2		39·4					39·6				40·4				40·0†								
10	,,	18	39·1			39·2		39·8					39·7				39·9				40·45				40·5			40·9†	
11	XI	21	39·3		38·45				39·5					40·7															Found dead after 9h. 29m., when the body-temperature was 38° C.
12	,,	22	39·8		39·26				38·7					38·7				39·6				39·5						39·6†	
13	XIII	25	39·8	39·4						39·0					39·4					41·4			41·27			41·25†			
14	,,	26	40·1	40·5						40·46					38·9					40·4			49·86						Found dead after 14h. 49m., when the body-temperature was 38·6° C. (Died between 14h. 42m. and 14h. 49m. after inoculation).
15	XVI	32	38·7		39·3				39·15										39·3				39·1						† Between 15h. 49m. and 18h. 38m. after inoculation.
16	XVIII	35	39·9		39·8				40·05					40·4				41·2				41·7†							
17	,,	36	39·8		39·4				39·8					39·2				39·6							40·2†				
18	XX	39	39·6		38·8				39·7										40·2										
19	,,	40	40·0		39·4				39·1										39·5							38·4†			Found dead 13h. 20m. after inoculation Body-temperature was then 35·6° C.

Note.—Wherever the symbol † is used in this Table, it means "died."

TABLE VI. (*a*).

SHOWING results of experiments (by feeding) on indigenous Birds.

Names of birds.	Date and time of feeding.	Infected food as placed in each cage.		Results.	Remarks.
		Description.	Quantity.		
Two Wekas ... ... (in one cage).	1888. Oct. 12th, 11.5 a.m.	Liver and heart-blood from rabbit No. 22 of Inoculation Series XI., Table III.	10 g.	Both were still alive on October 19th	They ate all the food at once.
Two Magpies ... (in one cage).	,, 11.10 a.m.	ditto ditto ditto	10 g.	One was found dead at 6.35 a.m. on October 13th (*i.e.*, between 18h. 50m. and 19h. 25m. after being fed). *P.M.*, *Positive.* The other was found dead at 1 p.m. on the same day (*i.e.*, between 25h. 25m. and 25h. 50m. after being fed). *P.M.*, *Positive.*	They ate all the food at once.
Two Laughing Jack-asses. (in one cage).	,, 11.15 a.m.	ditto ditto ditto	10 g.	One was found dead at 3.5 p.m. on October 17th (*i.e.*, between 122h. 45m. and 124h. 50m. after being fed). *P.M.*, *Negative.* The other was still alive on October 19th.	They had eaten all the food in five minutes.
Two Butcher-birds and one Blue Jay. (in one cage).	,, 11.20 a.m.	ditto ditto ditto	10 g.	One Butcher-bird was found dead at 6 a.m. on October 13th (*i.e.*, between 10h. 30m. and 18h. 40m. after being fed). *P.M.*, *Positive.* The other Butcher-bird was found dead at 9.20 a.m. on the same day (*i.e.*, between 21h. 10m. and 22h. after being fed). *P.M.*, *Positive.* The Blue Jay was found dead at 2.10 p.m. on the same day (*i.e.*, between 26h. 30m. and 26h. 50m. after being fed). *P.M.*, *Positive.*	They had eaten all the food in a quarter of an hour.
Two Gallahs ... ... (in one cage).	,, noon	ditto ditto ditto (mixed with 0·6 p.c. salt-solution and mashed up with crushed maize).	1 g. (excl. of salt-solution and maize).	One was found dead at 7.30 a.m. on October 14th (*i.e.*, between 37h. and 43h. 30m. after being fed). *P.M.*, *Positive.* The other was still alive on October 19th.	They had eaten about half in 2½ hours, and in 5 hours had eaten all.
Two Wonga Pigeons and one Bronze-wing Pigeon. (in one cage).	, 12.20 p.m.	ditto ditto ditto	1½ g. (as before).	One Wonga Pigeon was found dead at 12.48 a.m. on October 14th (*i.e.*, between 35h. 36m. and 36h. 28m. after being fed). *P.M.*, *Positive.* The Bronze-wing Pigeon was found dead at 12.30 p.m. on October 13th (*i.e.*, between 23h. 10m. and 24h. 10m. after being fed). *P.M.*, *Positive.* The other Wonga Pigeon was still alive on October 19th.	They had eaten about half in 2 hours, and in 4½ hours had eaten all.
Two Quail ... ... (in one cage).	,, 12.25 p.m.	ditto ditto ditto (a similar mixture mashed up with bread-crumbs).	½ g. (cf. above).	One was found dead at 6 a.m. on October 13th (*i.e.*, between 9h. 30m. and 17h. 35m. after being fed). *P.M.*, *Positive.* The other was still alive on October 19th.	They had eaten all in an hour.

Controls: A full-grown rabbit, fed upon cabbage-leaves infected with 1 g. of the same material as above (infusion in 0·6 p.c. salt-solution), died 33h. 50m. after being fed. *P.M.*, *Positive.*
A half-grown rabbit, fed upon cabbage-leaves infected with ½ g. contained in a similar infusion, died between 37h. and 42h. 50m. after being fed. *P.M.*, *Positive.*

TABLE VI. (*b*).

SHOWING results of further experiments (by feeding and inoculation) on the indigenous Birds surviving from the experiments, as detailed in Table VI. (*a*).

Names of birds.	Date and time of treatment.	Nature of treatment.	Quantity of the material used.	Results.	Remarks.
Two Wekas (kept, after treatment, in one cage).	Oct. 19th, 9.45 a.m. ,, 10.10 a.m.	One was inoculated with liver-blood from rabbit No. 34, Inoculation Series XVII., Table III. The other was fed upon liver from the same rabbit.	1 platinum loopfull (about 1-200th ccm.) 10 g.	Both were still alive on October 29th.	The one which was fed (separately from the inoculated one), ate all at once.
One Laughing Jackass	,, 10.20 a.m.	Fed on the same material...	7½ g.	Was still alive on October 29th.	In half-an-hour it had eaten all except a small piece, and in 3½ hours it had eaten all.
One Wonga Pigeon ...	,, 10.40 a.m.	Ditto ditto ditto (mixed with 0·6 p.c. salt-solution and mashed up with crushed maize).	1 g. (excl. of salt-solution and maize).	Was found dead at 7.15 a.m. on October 22nd (*i.e.*, between 59h. 20m. and 68h. 35m. after being fed). *P.M.*, *Positive*.	It had eaten all in half-an-hour.
One Gallah	,, 10.50 a.m.	Ditto ditto ditto	1 g. (as before).	Was still alive on October 29th.	In an hour it had eaten more than half, and in 3½ hours it had eaten all.
One Quail	,, 10.55 a.m.	Ditto ditto ditto (a similar mixture mashed up with bread-crumbs).	½ g. (cf. above).	Was found dead at 12.25 p.m. on October 20th (*i.e.*, between 25h. 5m. and 25h. 30m. after being fed). *P.M.*, *Positive*.	In an hour it had eaten nearly all, and in 2 hours had finished all.

Controls: (*a*). Two half-grown rabbits, in one hutch, were fed upon cabbage-leaves infected with 1½ g. of the same material as above (infusion in 0·6 p.c. salt-solution) for the two. *One* died between 22h. 45m. and 24h. 40m. after being fed. *P.M.*, *Positive*. The other died between 94h. 25m. and 98h. 35m. after being fed. *P.M.*, *Negative*.

(*b*). A half-grown rabbit, inoculated with one platinum loopfull (about 1-200th ccm.) of the liver-blood (as used above), died between 13h. 20m. and 21h. 35m. after being fed. *P.M.*, *Positive*.

TABLE VI. (*c*).

SHOWING results of further experiments (by inoculation) on the indigenous Birds surviving from the experiments, as detailed in Table VI. (*b*).

Names of Birds.	Date and time of inoculation.	Inoculation (by way of injection) with	Results.	Remarks.
Two Wekas (in one cage).	Oct. 29th, 10.45 a.m. ,, 10.50 a.m.	1-16th ccm. (1 minim) of heart-blood from a rabbit dead between 9h. 5m. and 17h. 15m. after inoculation with a virulent broth-culture of the microbe of chicken-cholera. ditto ditto ditto	Both remained alive. For further treatment see Table VI. (*d*).	
One Laughing Jackass	,, 10.40 a.m.	ditto ditto ditto	It was found dead at 2.5 p.m. on November 1st (*i.e.*, between 71h. 20m. and 75h. 25m. after inoculation). *P.M.*, *Positive*.	A half-grown rabbit, inoculated with a good platinum loopfull of heart-blood from this jackass, died between 11h. 15m. and 12h. 35m. after inoculation. *P.M.*, *Positive*.
One Gallah	,, 11.5 a.m.	ditto ditto ditto	It was found dead at 7.15 a.m. on October 30th (*i.e.*, between 11h. 40m. and 20h. 10m. after inoculation). *P.M.*, *Positive*.	

Control: A full-grown rabbit inoculated with a like quantity of the same material, died between 11h. 15m. and 19h. 40m. after inoculation. *P.M.*, *Positive*.